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The Microbiological Safety of Fresh Produce in Lebanon- A holistic "farm-to-fork chain" approach to evaluate food safety, compliance levels and underlying risk factors

Faour-Klingbeil, Dima

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The Microbiological Safety of Fresh Produce in Lebanon- A holistic “farm-to-fork chain” approach to evaluate food safety, compliance levels and underlying risk factors

By

Dima Faour-Klingbeil

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ABSTRACT

The consumption of unsafe fresh vegetables has been linked to an increasing number of outbreaks of human infections. In Lebanon, although raw vegetables are major constituents of the national cuisine, studies on the safety of fresh produce are scant. This research employed a holistic approach to identify the different stages of the food chain that contribute to the microbiological risks on fresh produce and the spreading of hazards. A thorough analysis of the institutional and regulatory framework and the socio-political environment showed that the safety of local fresh produce in Lebanon is at risk due to largely unregulated practices and shortfalls in supporting the agricultural environment as influenced by the lack of a political commitment.

Microbiological analysis showed that the faecal indicator levels ranged from <0.7 to 7 log CFU/g (*Escherichia coli*), 1.69-8.16 log CFU/g (total coliforms) and followed a significantly increasing trend from fields to the post-harvest washing area. At washing areas, *Salmonella* was detected on lettuce (6.7% of raw vegetables from post-harvest washing areas). This suggested that post-harvest cross-contamination occurs predominantly in the washing stage.

At retails, a combination of observation and self-reported data provided an effective tool in assessing knowledge, attitudes and practices. It showed that the food safety knowledge and sanitation practices of food handlers were inadequate, even among the better trained in corporate-managed SMEs. Overall, the microbiological quality of fresh-cut salad vegetables in SMEs was unsatisfactory. The link between *Staphylococcus aureus* and microorganism levels on fresh salads vegetables and the overall inspection scores could not be established. On the other hand, inspection ratings on individual components, e.g., cleanliness and cross-contamination preventive measures showed significant correlation with *Listeria* spp. levels. Together, results confirmed that inspection ratings don't necessary reflect the microbiological safety of fresh vegetables and that the application of control points of risk factors that likely to contribute to microbial contamination in the production environment are essential.

The washing methods were limited in their effectiveness to reduce the contamination of parsley with *Salmonella*. In general, the pre-wash chopping and storing of parsley at 30ºC reduced the decontamination effect of all solutions, including sodium dichloroisocyanurate which was reduced by 1.3 log CFU/g on both intact and chopped leaves stored at 30ºC.

In such conditions, the transfer rate of *Salmonella* from one contaminated parsley to subsequently chopped clean batches on the same cutting board(CB) recorded 60%-64%. Furthermore, the transmission of *Salmonella* persisted via washed CBs stored at 30°C for 24 h. It is recommended to keep parsley leaves unchopped and stored at 5ºC until wash for an optimum decontamination effect and to apply vigilant sanitation of CBs after use with fresh produce.

This research presented important data for quantitative risk assessment for *Salmonella* in parsley and useful descriptive information to inform decision-makers and educators on microbial hazards associated with fresh produce in Lebanon. It also highlighted the risks areas that require urgent interventions to improve food safety. Considering the complex institutional and political challenges in Lebanon, there is an obvious need to direct development programs and support towards local agriculture production, effective education strategies and growing awareness of consumers and stakeholders on food safety related risks.

DEDICATION

This thesis is foremost dedicated to my dear parents, my husband and our children for their endless love, support and encouragement

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AUTHOR'S DECLARATION

At no time during the registration of the Degree of Doctor of Philosophy has the author been registered for any other University award. Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

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The following activities and programmes have been undertaken:

Scientific publishing

1. Understanding the routes of contamination of ready-to-eat vegetables in the Middle East. *Food Control* (2016), 62:125-133. http://dx.doi.org/10.1016/j.foodcont.2015.10.024

2. Investigating a link of two different types of food business management to the food safety knowledge, attitudes and practices of food handlers in Beirut, Lebanon. *Food Control* (2015), 55: 66-175. http://dx.doi.org/10.1016/j.foodcont.2015.02.045

3. Prevalence of antimicrobial resistant *Escherichia coli* from raw vegetables in Lebanon. *The Journal of infection in the developing countries* (2016), 10(4):354-362. doi:10.3855/jidc.7745.

4. The influence of pre-wash chopping and storage conditions of parsley on the efficacy of disinfection against *S*. Typhimurium. *Food Control* (2016), 65:121-131. http://dx.doi.org/10.1016/j.foodcont.2016.01.019

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6. The transfer rate of *Salmonella* Typhimurium from contaminated parsley to other consecutively chopped batches via cutting boards under different food handling scenarios *Food Research International Journal* (Sept.2016), in press. http://dx.doi.org/10.1016/j.foodres.2016.09.001

Oral Presentations in conferences

1. First Nutrition and Food Technology Conference, USJ, Medical Sciences Campus, Beirut,16-17 May 2016. *"Understanding the routes of contamination to fresh produce - Lebanon"*

2. The International Association of Food Protection's 12th European Symposium on Food Safety, May 11-13, 2016, Greece (selected among finalists for award competition)

“*The Influence of Pre-wash Chopping and Storage Conditions of Parsley on the Efficacy of Disinfection against Salmonella Typhimurium*"

3. The Lebanese Association for the Advancement of Science (LAAS) International Science Conference, March 2015: “*Qualitative risk assessment in relation to the microbial quality of fresh produce from farms to market”*

4. World Health Organization (WHO) - Food Safety Regional Meeting, Amman, Jordan 2015. **(Temporary advisor and Speaker)** *“The importance of food safety knowledge, attitudes and Practices (KAP) among food handlers in the region”.* Contributed to recommendations for the Summary report on the “Regional meeting on food safety” (http://applications.emro.who.int/docs/IC_Meet_Rep_2015_16336_EN.pdf?ua=1)

5. 3rd FOODSEG Symposium in Novi Sad, Sebia- EU experts meeting, *“Safe Food for Europe – recent scientific and regulatory developments”,* 24-25 April 2014. Participated in group discussions explaining my research project for potential collaboration

6. The Lebanese Association for the Advancement of Science (LAAS) International Science Conference, March 2014. “*Food safety in Beirut- Links between microbiological quality of fresh vegetables and knowledge, attitudes and practices of food handlers*”

7. Centre for Agricultural and Rural Sustainability (CARS) Workshop, University of Plymouth, June 2013 “*Food safety knowledge, attitudes and practices of food handlers in Beirut and their relationship to microbiological quality of fresh vegetable*”. (winner of oral presentation).

Relevant Trainings

- **XII workshop on Rapid methods and automation in food microbiology,** The Universitat Autonoma de Barcelona-Centre Especial de Recerca**,** Spain**,** November 2013.Attended workshop and practical trainings in detection methods

-**Hands on training on molecular biology techniques,** Plymouth University, June 2013

Attended

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Signed: Dima Faour-Klingbeil

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APC	Total plate counts
ASAIL	Action for Sustainable Agro-industry in Lebanon
CDC	Centre for Disease Control and Prevention
CFU	Colony forming units
CITI	Collaborative Institutional Training Initiative
CSPI	Center for Science in the Public Interest
EFSA	European Food Safety Authority
ENPARD	European Commission’s initiative for agriculture and rural development
EU	European Union
FSE	Food service establishment
GAP	Good Agricultural Practices
GHP	Good Handling Practices
HACCP	Hazard analysis of critical control points
IDAL	Investment Development Agency for Lebanon
KAP	Knowledge, Attitudes and Practices
L. monocytogenes	*Listeria monocytogenes*
LFSA	Lebanese Food Safety Agency
MENA	Middle East and North Africa
Min.	Minute
MoA	Ministry of Agriculture
MoE	Ministry of Economy
MoPH	Ministry of Public Health
MoT	Ministry of Tourism
NaClo	Sodium hypochlorite

NaDCC	Sodium dichloroisocyanurate
NTWG	National Technical Working Group
PBC	Perceived Behavioural Control
ppm	Parts per million
PW	Peptone water
QCC	Quality Control and Certification
RTE	Ready-to-eat food
S	Second
S.aureus	*Staphylococcus aureus*
S. Typhimurium	*Salmonella*
SME	Small and medium-sized food service establishments
SPS	Sanitary and phytosanitary measures
TC	Total coliforms
Tr	Transfer rate
TBT	Technical Barriers to Trade
TSB	Tryptone soya broth
TSBN	Tryptone soya broth supplemented with nalidixic acid
US	United states
USFDA	US Food Safety Modernization Act

1. General introduction and literature review

1.1. Background

To date, despite the numerous research and continuous efforts directed towards updating legislations, enactment of laws, strict regulations and enforcement, foodborne illnesses still persist as a global public health issue. Food outbreaks are sometimes traced back to known pathogens and many are traced to unknown aetiology or newly emerged pathogens (WHO, 2008; CDC, 2013). Of major concerns are the emergence of antimicrobials resistant pathogens (O'Brien, 2002; Schwaiger et al., 2011) and the increasing implications of fresh produce in food outbreaks in many parts of the world (Olaimat & Holley, 2012; Oliveira et al., 2012). According to WHO/FAO (2008a), leafy vegetables were ranked as the highest priority in terms of microbiological hazards, and have been associated with high numbers of illnesses in at least three regions of the world: United states (US), Asia and Europe. The intensified global trade of fresh produce, driven by increased demands, improved agricultural production technology, and changes in consumers' dietary habits, makes fresh produce available all year around and potentially increases human exposure to a wide array of foodborne pathogens as local risks spread relatively fast into international markets (Gereffi & Lee, 2009). As many stakeholders are involved in the distribution system, this would eventually result in slow epidemiological investigations that may extend to weeks before detecting the source (produce type) of the reported illnesses (FDA/CFSAN, 2001). As such, there is a wide recognition of the economical and health impact that global markets could pose and the importance of raising the bars of standards in exports markets particularly in the developed countries. For instance, a number of cases of *Escherichia coli* (*E. coli)* and *Salmonella* in the US have been associated with the consumption of domestic and imported foods including fruit and vegetables (FDA, 2011b). Consequently, the fresh produce industry has witnessed internationally a transition from food safety and quality control of fresh produce to a food safety assurance and prevention approach to limit contamination (Gil et al., 2015). This approach requires a system that is science-based and uses risk analysis to focus preventive efforts and risk management on the areas or processes that are most likely to cause foodborne illnesses (Shames, 2008).

In this regard and in response to the spate in infection rates attributed to food poisoning (CDC, 2010), the US Food and Drug Administration (USFDA) has been mandated through the Food Safety Modernization Act (FSMA) to establish science-based minimal standards for the safe production and harvesting of produce including minimally-processed fruits and vegetables. The law entails that the FDA develops safe agronomic practices that must be adopted by those who export to the US (FDA, 2011c). Similarly, the European Food Safety Authority's Panel on Biological hazards (EFSA's BIOHAZ Panel) identified a range of environmental risk factors and indicated that the edible portion of crops subjected to spraying prior to harvest and direct application of fertilizers are at high risk of *Salmonella* and norovirus contamination (EFSA, 2014). Eventually, EFSA members proposed that, while each farm environment is different, the primary objectives for producers should include good agricultural practices (GAP), good hygiene practices (GHP) and good manufacturing practices. On the other hand, most developing countries lack the capacity to meet required standards due to economic constraints, lack of technical skills and capability to verify compliance to standards throughout the value chain (Henson & Humphrey, 2009; Lee et al., 2012a), and this is a common case in most countries of the Middle East and North Africa (MENA) region, including Lebanon (FAO, 2012b).

In 2001, 27% of food exports from Egypt, Jordan, Lebanon and Syria to the United States were rejected by the USFDA due to non-compliance with the U.S. safety measures (filth, microbiological contamination, greater than permitted levels of pesticide residues or food additives (CSPI, 2005). Not only external factors, such as population growth, climate change, poverty and technical incapacities act as impediments to compliance to safe practices, but also rather poor internal decisions for effective planning of resources and support mechanisms for ensuring consumers health protection are basically prominent factors that drive farmers in some regions of the developing countries to use untreated wastewater for irrigation and processing of vegetables, misuse of pesticides and fertilizers (Aiat Melloul & Hassani, 1999; Hanjra et al., 2011; Markou & Stavri, 2005).

To date, this is an important issue that has not been studied enough in the region, neither its implications particularly on the safety of fresh produce as it reaches consumers.

1.2. Fresh produce-related outbreaks: A growing concern

The incidences of food outbreaks linked to fresh produce have increased worldwide in the last two decades while new ones continue to emerge (Newell et al., 2010). According to the Centre for Science in the Public Interest (CSPI)'s database, fresh produce that are often eaten raw cause more foodborne illness than any other single category of food (CSPI, 2015); of 5,000 foodborne illness outbreaks, fruits and vegetables caused nearly 21 % of the associated illnesses between 1990 and 2004 (CSPI, 2005). The US and European Union (EU) have reported a total of 377 and 198 produce-associated outbreaks, respectively for the period 2004-2012 (Callejón et al., 2015).

The surveillance report from 1992 to 2008 in England and Wales showed a general decline in foodborne outbreaks indicating that complying with effective control measures is crucial to minimizing the risk of foodborne infection (Gormley et al., 2011). Similarly, the Centre for Disease Control and Prevention (CDC) in the States indicated further decline in the incidences of bacterial foodborne illnesses in 2003, that was attributed to continuous attempts to attain the national health objectives for reducing the incidence of foodborne infections by 2010 (CDC, 2004). Nonetheless, recent review of foodborne illnesses in the US from 2004-2013 showed that vegetables that are often consumed raw, e.g., cucumbers, pepper and cilantro, continue to cause more illnesses than any other food single category of food, and that many outbreaks would remain unsolved and untraced, in some cases for 3 years (CSPI, 2015). For instance, from 2012 until 2015, the investigations on 11 farms in Mexico linked the Cyclosporiasis outbreaks caused by Cilantro to the Mexican fields in Puebla that were littered with human faeces, which led to banning imports until documentation on growing practices are presented. Several other recent incidences of outbreak shed the light on the growing trend in international trade of fresh produce and its contribution to their occurrences particularly when the exports country apply lower food safety standards (Newell et al., 2010). The rise in reported cases is thought to be attributed to increased awareness on the consumption of fresh vegetables as a result of promotional campaigns for its health benefits (Mercanoglu Taban & Halkman, 2011), improvements in outbreak investigations and efficient detection methods in surveillance systems and DNA finger printing in the developed world and in countries with well-developed epidemiological surveillance systems which are still ineffective or unavailable in the developing world (Beuchat, 2002; FAO/WHO, 2008), cross national trades of fresh produce, modifications in agronomic practices and technologies, and awareness of epidemiologists on the implication of raw vegetables as potential vectors (Beuchat, 2002; Powell et al., 2004). Futhermore, it is thought that the number fresh produce-related outbreaks are underestimated as data may not reflect what occurs in sporadic cases, in addition, countries vary in their investigations systems and sensitivity of applied analysis (O'Brien, 2002).

There is a wide spectrum of human pathogens that can be associated with fresh vegetables which include *E. coli* O104:H4, *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella,* viruses (hepatitis A virus, norovirus) and parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*) (Jung et al., 2014). However, *Salmonella enterica* and *E. coli* are the two major species encountered in large outbreaks of foodborne illness associated with fresh produce and have been traced to a wide variety of produce, including lettuce, salads, melons, sprouts, tomatoes, and many fruit- and vegetable-containing dishes (European Commission, 2002; Buck et al., 2003; Yaron & Romling, 2014; Todd & Greig, 2015), with low infectious dose fewer than 40 viable cells for *E. coli* O157 (Strachan et al., 2005).

Between 1990-2005, norovirus and *Salmonella* emerged as common agents of produce-related outbreaks followed by *E. coli*, at 40%, 18% and 8%, respectively (DeWaal & GBhuiya, 2009). *Salmonella* was commonly responsible for produce outbreaks accounting for nearly half of the bacterial outbreaks in the US and the EU, and *E. coli* was the second most common pathogen identified as the cause of multi-state outbreaks in the US (Sivapalasingam et al., 2004).

1.2.1. The implication of *Salmonella* in large outbreaks of fresh produce

Salmonella is a genus of rod-shaped (bacillus) gram-negative bacterium of the Enterobacteriaceae family. The two species of *Salmonella* are *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into six subspecies that include over 2500 serovars which are identified as causative agents to diarrheal illness in humans (Su & Chiu, 2007) and considered to be potentially pathogenic (Jay, 2000).

Salmonella serovars can be divided into two main groups, typhoidal and non-typhoidal *Salmonella*. Non-typhoidal serovars are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and they can be transferred between humans and other animals (Zoonotic). Typhoidal serovars include *Salmonella* Typhi and *Salmonella* Paratyphi A, which are adapted to humans and do not occur in other animals.

About 2,000 serotypes of non-typhoidal *Salmonella* are known. Most cases of invasive non-typhoidal *Salmonella* infection are linked to *Salmonella* Typhimurium (*S.*Typhimurium) or *Salmonella* Enteritidis (*S.* Enteritidis) (Feasey et al., 2012).

The biochemical identification of *Salmonella* isolates is typically combined with serological confirmation involving agglutination of bacterial surface antigens with *Salmonella*-specific antibodies. These include O lipopolysaccharides on the external surface of the bacterial outer membrane, H antigens associated with peritrichous flagella and the capsular (Vi) antigen, which occurs only in *S*. Typhi, *S.* Paratyphi C, and *S*. Dublin. (D'Aoust, 2001).

Animals and birds are the natural reservoirs of *Salmonella*, hence this pathogen remains well known in the meat and poultry industries (D'Aoust, 2001) and frequently implicated in salmonellosis outbreaks linked to poultry and other meat products, eggs and dairy products.

Nonetheless, several human infections were linked to *Salmonella* serotypes isolated from fresh produce (Harris et al., 2003), including alfalfa sprout, lettuce, fennel, cilantro, cantaloupe, unpasteurized orange juice, raw tomatoes, melon, mango, celery and parsley (Lapidot et al., 2006) with infectious dose ranging from 10 to 10^5 (IFT/FDA, 2001). Faeces of infected humans or animals can leach into the food and water system through sewage overflows, dysfunctional sewage systems or waste water and contaminated agricultural runoff (CDC, 2009) or by insects and other living creatures (Jay, 2000), however sources extended as well to cross-contamination from raw meat, poultry, or eggs (IFT/FDA, 2001).

In the US, eight lettuce-associated outbreaks were associated with foodborne pathogens that included *Salmonella* (Sivapalasingam et al., 2004). Additionally, large multi-states outbreaks of salmonellosis have been attributed to consumption of raw tomatoes; one involved *Salmonella* Javiana in 1992, another, *Salmonella* Montevideo in 1993, and a third in 2000 involved *Salmonella* Baildon.

Table 1.1 shows recent incidences of salmonellosis caused by fresh produce.

1.2.2. Foodborne illnesses and challenges in Lebanon

There is a dearth of studies on the microbiological quality of fresh vegetable in Lebanon and related foodborne illnesses. The available information does not provide a comprehensive picture on the fresh produce safety. Most of the cases in Lebanon communicated in local news and reported in the surveillance system (PulseNet, 2011-2012) are at large those linked to raw meat or foods of animal origins. Although the notification of a number of foodborne diseases is obligatory, yet it is considered underreported and data may not be a true reflection of actual numbers (Ghosn et al., 2008). In 2001, 17 episodes of food poisoning were identified among the 92 reported ‘food poisoning’ cases. After investigation, it became evident that there were 112 cases in total, of which 84 were hospitalized. The factors identified were raw meat, cooked meat, sandwiches, sweets, other and unspecified (FAO/WHO, 2005a). In another incident, in 2004, more than 30 employees in a local bank in Beirut showed symptoms of gastroenteritis and after investigation the food-borne outbreak was caused by Salmonellosis related to infected raw chicken (Hanna et al., 2009). From 2009 to 2010, data from the Ministry of Public Health (MoPH) showed a twofold increase in incidences of food poisoning in Lebanon indicating 247 and 483 cases, respectively. The highest rate of occurrence was detected among people aged 20-39 years in both years and especially in the months July and August (Figure 1.1).

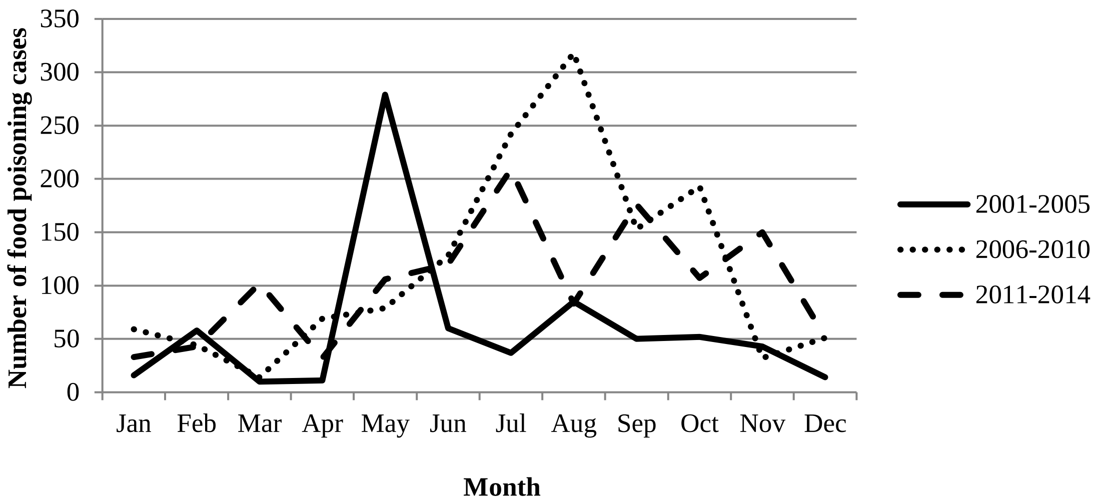


Figure 1.1. The number of food poisoning incidences in Lebanon during the period of 2001-2014 presented as average over three "five years" time periods (MoPH, n.d.)

Lebanon is currently facing many governance challenges; the political-economic structure negatively impacted on the realization of an overall effective administration (El-Saad, 2001). The implementation of an effective food safety system, activating or updating existing legislations, and improving accountability is poor (Kamleh et al., 2012). The food safety control is based on end-product analysis instead of a risk-based system that requires overlooking several nodes throughout the food chain (Abou Ghaida et al., 2014). Consequently, the production and distribution of fresh vegetables particularly to local markets are currently characterised by insufficient quality and safety control as well as deficient regulations (CTCS, 2010). Both are essential to minimize risks that might emerge from unsafe practices. Along the same lines, Zurayk & Abou Ghaida (2009) reported on the limited control over the safety of local produce destined for the domestic market. Nevertheless, there exist incentives and supportive programs to facilitate farmers' access to international markets and which require commitment to food safety standards (DAI, 2015).

Figure 1.2. Sewage channeled into a river stream used for irrigation in Bekaa

More concerning is the pollution of main sources of water for irrigation by sewage and industrial effluents, especially the Litani River and the Litani-Awali complex (Houri & El Jeblawi, 2007). Most rivers in Lebanon are contaminated with raw sewage contamination and leakage from uncontrolled dumpsites, including the largest river, Litani river, and aquifers (MoE, 2001) (Figure 1.2). In 2006, only 4 million m^3 of 310 million m^3 annually generated wastewater in Lebanon were treated. Half of this amount was channelled for agricultural use and the agricultural sector relies to some degree on untreated wastewater for irrigation (FAO, 2008).

Local data on the rate of consumption of raw vegetables is scarce, but it seems it was on the rise in the last decade (Figure 1.3). In parallel, the Lebanese cuisine is famous with its richness in fresh leafy vegetables as major constituents of traditional salads and cold appetizers, which with their large surfaces are likely to present high risks as vehicles of pathogens (WHO/FAO, 2008). In this context, a report dated back to 1998 indicated that the prevalent diseases implicated by polluted water in Lebanon are typhoid, hepatitis, and dysentery and their incidences correlated with the periods of irrigation of vegetables crops with polluted water (Sarginso et al., 1998). Therefore, incidences of food outbreaks such in countries renowned for their strict standards have possibly occurred but remained unreported in the developing countries (FAO/WHO, 2008), including Lebanon.

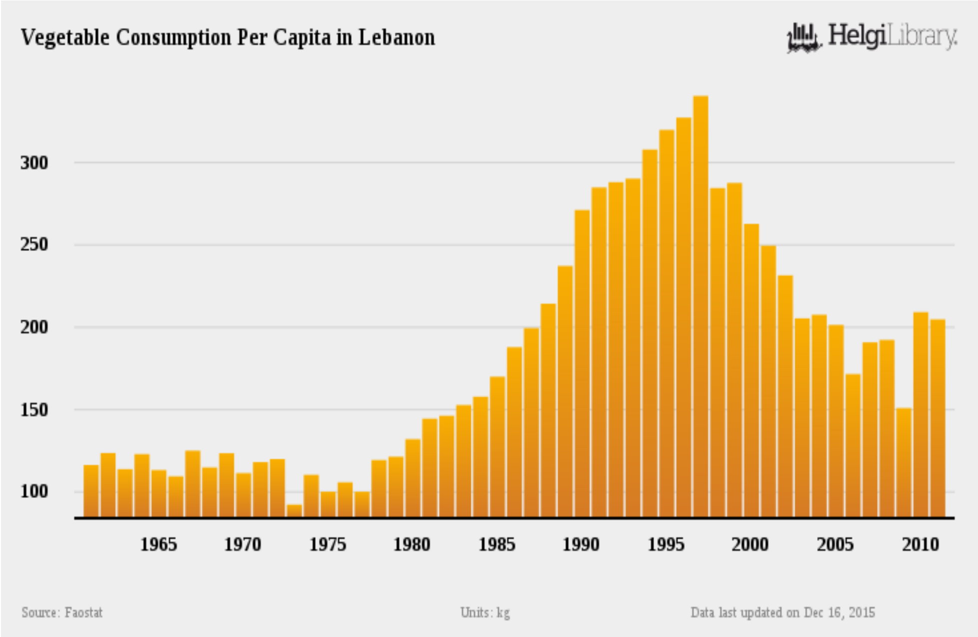


Figure 1.3. Vegetable consumption per capita in Lebanon. Source (FAOSTAT)

1.2.3. Contamination routes to fresh produce and persistence of *Salmonella*

There are various pathways whereby fresh produce is subjected to bacterial hazards that can take place at any step from the farm-to-the fork during harvesting, handling, transportation, processing and packaging; as such, these commodities that were deemed to be harmless are recognized to be potentially hazardous and can lead to deadly infections as in the case of the most renowned 2011 sprout outbreak (Buchholz et al., 2011).

Salmonella including *E. coli* O157:H7, *Campylobacter jejuni*, *Vibrio cholerae*, parasites and viruses are more likely to contaminate fresh produce through the mechanism of transfer via the faecal–oral route, e.g., vehicles such as raw or improperly composted manure, sewage contaminated water used for irrigation, animals accessing crops or contaminated wash water (Franz, 2005; Sivapalasingam et al., 2004).

Investigations of 1998-1999 on *S.* Baildon outbreak of tomatoes in Florida reported that contamination was suspected to have occurred on farm caused by domestic or wild animals (Cummings et al., 2001). Contaminated soil and polluted water used for irrigation and washing of fresh produce remains a growing concern as a primary contamination vector of fresh produce at source and is common in the developing world (Pachepsky et al., 2011). The 1993 large multistate outbreak caused by *S*. Montevideo on tomatoes was traced back to contamination of water bath used by packer (Lund & Snowdon, 2000). Infected livestock or improperly treated effluents, such as sewage from treatment plants can transmit pathogens such as Norovirus, Hepatitis A, or bacterial pathogens to both surface and ground water sources (Beuchat, 1998) which can be internalized to the inner tissues of plants (Bova & Walker, 2000). It is well established that pathogens could survive for extended periods of time in water (Balzaretti & Marzano, 2013). The survival of both *E. coli* O157:H7 and *S*. Typhimurium in water at 4 and 22ºC declined by only 1 log after 28 days (Chang & Fang, 2007) indicating post-harvest stages as source of hazards.

It is documented that *S.* Typhimurium can persist in the soil for extended periods (203 to 231 days) particularly when treated with poultry compost (Islam et al., 2004). This pathogen was also capable of surviving on vegetables and in soil samples contaminated by irrigation water for several months (Islam et al. 2004). Additionally, *S*. Typhimurium survived for at least 100 days on parsley or basil (Kisluk et al., 2012; Kisluk et al., 2013). Earlier studies showed that *Salmonella* could survive on fresh produce such as on alfalfa sprouts for 10 days at 58ºC (Jaquette et al., 1996) and in optimum conditions this pathogen could grow on tomatoes stored for 7 days at 20ºC (Zhuang et al., 1995).

The manure is a major contamination factor on farms as agricultural fields is commonly fertilized with manure that comes from chicken, beef, and pork farms. Besides, most of farms use medically important antibiotics in animal feeds, which possibly further contribute to increased pathogens, particularly, antibiotic resistant pathogens in produce (Heuer et al., 2011).

Table 1.1. Incidences of fresh produce-related outbreaks (2000-2015)

Most recent fresh produce-related outbreaks/Country	Type of produce/Origin	Note
2012-2015: annual outbreaks of Cyclosporiasis *(Cyclospora cayetanensis*) in the US	Cilantro / Mexico	Investigations in July 2015 found that poor hygienic conditions for farm workers were most likely the cause of those outbreaks (CSPI, 2015)
2015: *Salmonella* Poona outbreak, 767 people infected from 36 states	Cucumbers from Mexico	Unknown
2014: *Salmonella* Newport outbreak in 2014 sickened 257 patients in 29 states and the District of Columbia.	Cucumbers / unidentified source	The pathogen was assumed to be linked to the application of manure (CSPI, 2015)
2012: *S.* Typhimurium and *Salmonella* Newport in 2012, sickened 261 people in 24 states, 3 deaths and 94 hospitalizations.	Cantaloupes	An inspection found unsanitary conditions in the farm's processing shed. (CSPI, 2015)
2011: Major EHEC O104:H4 outbreak in Germany resulting in 3000 cases with bloody diarrhoea, 852 cases of haemolytic uremic syndrome, and 53 deaths	sprouted fenugreek seeds/ traced to shipment of seeds from Egypt to Germany	Mora et al. (2011)
2008: *Salmonella* Saintpaul sickened 1,442 people in 43 states.	Jalapeño and serrano peppers and pepper products (e.g., salsa)/ Mexico	Contaminated irrigation water was suspected (CSPI, 2015)
2006: Multi-state outbreak of *E. coli* O157:H7, 205 sickened and 3 deaths	Spinach	Contaminated fields by swine faeces. (CDC, 2006)
2005: *S.* Typhimurium in Finland, affecting 60 people	Lettuce / iceberg imported from Spain	Takkinen et al. (2005)
2005: one outbreak of *E. coli* O157:H7 in Sweden, 120 cases	Lettuce / iceberg	Irrigation from a stream was suspected (Söderström et al., 2005)
2005: one outbreak of *E. coli* O157:H7 in USA, more than 12 sickened	Parsley	FSnet (2005)
2004: *Salmonella* Thompson in Norway, some cases in Sweden, 20 sickened	Rucola/Rocket imported from Italy	Nygård et al. (2008)
2004: *Salmonella Newport* in UK, 375 sickened	Lettuce imported from Spain	Gillespie (2004)
2001: *Listeria* affected 147 people in 28 states and caused 33 deaths	Cantaloupes	The outbreak was linked to unsanitary conditions at the packing facility on the farm (CSPI, 2015)
2000: two *S.* Typhimurium outbreaks in England, Wales, Scotland, Ireland, Germany and Netherlands sickening 392 people	Imported lettuce/Iceberg	Crook et al.(2003)
2000: *Cyclospora cayetanensis* outbreak in Germany, 34 sickened people	Imported lettuce (unidentified)	Probably contaminated through fertilization with human waste or fecal contaminated water used to irrigate crops (Döller et al., 2002)

Several other points along the farm-to-fork route can potentially lead to the bacterial contamination of raw vegetable. Investigations of several foodborne illnesses linked to fresh produce indicated that agricultural workers were identified as the source of the pathogen. However, post- harvest treatment of fruits and vegetables including handling, storage, transportation, and cleaning may also lead to cross-contamination of the produce from other agricultural materials or from the workers and food handlers (NACMCF, 1999).

1.2.4. Biofilm formation on fresh produce

Survivals of pathogens for long periods are attributed to attachment and colonization on the surfaces of growing plants. As human pathogens are attached on the surfaces of vegetables particularly on cut surfaces, they are able to colonize and form biofilms (Escudero et al., 1999; Fett, 2000; Beuchat, 2002; Ells & Hansen, 2006; Tang et al., 2012). The localization of bacteria in cracks and crevices on the plant and the formation of biofilms or integration of foodborne pathogens into existing biofilms on the plant's surfaces are among conceivable explanations for the ineffectiveness of disinfectants and sanitizers. (Koseki et al., 2001b; Ölmez & Temur, 2010).

A biofilm is a biologically active matrix consisting of sessile microbial communities of cells in association with a substratum and frequently embedded in an organic polymer matrix of microbial origin (Garrett et al., 2008). Water is the major component of biofilms (up to 97%) whereas bacterial cells constitute 35% of the dry weight (Yaron et al., 2014). Adherence to a substrate depends on physical appendages and extra-cellular polymeric substances. The essential requirements for biofilm growth are the microbes themselves and a substrate (Garett et al, 2008). In the initial stage, the adhesion is weak, reversible and characterized by nonspecific binding that is often affected by hydrophobic and electrostatic interactions. In the successive stage of binding, a strong irreversible attachment might take place (De Oliveira et al., 2014; Dunne, 2002) and is referred to as ‘firm’ attachment for the unattainable removal of the attached bacteria. In many symbionts, the second biding stage involves bacterial cellulose fibres (Laus et al., 2005), for *Salmonella*, the ability to form biofilm on several types of fresh vegetables is well documented and this means that consumers are at risk when biofilm would limit and preclude the disinfecting efficacy of various sanitizers (Koseki et al., 2001b; Ölmez & Temur, 2010). In Gram-negative bacteria, flagella, fimbriae types I, IV and curli, play major role in the primary stages of adherence to enable bacteria motility to surfaces and to counteract hydrophobic repulsions. However, adhesion can be influenced by different physicochemical properties of these surfaces, and *Salmonella* uses fimbriae and produces cellulose as the main matrix components of biofilms (De Oliveira et al., 2014). Although motility helps the process, it does not seem to be an essential requirement for adhesion. Annous et al. (2004) showed instant formation of biofilms when *Salmonella* cells were inoculated onto the melon surface. Fibrillar material was visible after just two hours, and cells were embedded in an extracellular polymeric material after 24 h. These results indicate that a human pathogen can form a biofilm on plant tissues and that biofilm formation may be responsible for the increased resistance of attached bacteria to applications of sanitizers (Annous et al., 2004).

The strength of bacterial adherence is dependent on many factors which include inoculum size, contact time, the host plants, strains and species. For instance, *S. enterica* serovars showed varied affinity to basil, lettuce or spinach; *S.* Senftenberg and *S.* Typhimurium showed higher attachment compared with *S.* Agona or *S.* Arizonae (Berger et al., 2010). In another study, Shaw et al. (2011) observed three *Salmonella* serovars attached to tomato fruits sand noted that serovars Senftenberg and Typhimurium adhered to the fruits in an aggregative pattern, whereas serovars Thompson adhered in a diffuse pattern. Additionally, *S.* Typhymurium adhered much strongly to cucumbers than *L. monocytogenes* (Reina et al., 2002). On the other hand, while *E. coli* attached stronger to cut surfaces of lettuce, *Pseudomonas fluorescens* cells adhered preferentially to the intact areas, and *S*. Typhimurium attached equally to both, cut and intact surfaces (Takeuchi et al., 2000). Other authors suggested that surface properties alter the attachment of cells irrespective of strains and that adherence is reduced on waxy materials of uncut surfaces, unlike hydrophilic bruised and cut surfaces that enhanced cells attachment (Ells & Hansen, 2006).

A recent study that examined the adhesion and persistence of *S.* Typhimurium and its biofilm-deficient isogenic mutant on parsley showed that EPS and curli were irrelevant to strength of attachment. After a week of storage, the biofilm-producing strain survived chlorination significantly better than the biofilm-deficient mutant. However, as the recovery of the mutant cells was still high, authors indicated the biofilm matrix is not likely to be the single mechanism responsible. Other factors underline the persistence of *Salmonella* after chlorination and other mechanisms, such as penetration to plant tissue or pre-existing biofilms, or the production of polysaccharides other than cellulose that provide additional protection to cells (Lapidot et al., 2006).

On the other hand, the development of biofilms is also likely to happen on containers of harvested crops when they are not effectively cleaned and sanitized and remain in contact with pathogens for sufficient time during display or transportation (Beuchat, 2002). Consequently, fresh produce could therefore be contaminated with surfaces harbouring these biofilms (Helke & Wong, 1994). Overall, there is no one single clear mechanism that explain the bacterial attachment. It is rather influenced host plants, bacterial species and environmental conditions (Yaron et al., 2014).

There is a wide consensus on the internalization theory and that *Salmonella* spp. is capable of proliferating to high levels on or within the plant (Schikora et al., 2012). It is likely that bacteria can enter the plant and move through it passively, being transported via the mass flow of water entering the plant and moving within it (Deering et al., 2012). Besides, the penetration within internal organs following internalization is demonstrated to protect bacteria from direct contact with the disinfectant.

Some results have suggested that *Salmonella* has the ability to enter fruits, and possibly other plant parts, through cuts, where they may persist (Guo et al., 2002) as well as by invading the seeds through contaminated soil, and later growing to 3.5 log during germination (Singh et al., 2005). Recently, it was shown that *S.* Typhimurium can penetrate the epidermis of iceberg lettuce leaves through open stomata in a process that involves flagellar motility and chemotaxis (Kroupitski et al., 2009). In this case, over chlorinated water may reduce the microorganisms' levels on the surface but would not completely eliminate internal populations of *Salmonella* (Zhuang et al., 1995).

Nonetheless, there is still no general agreement on the findings of internal uptake of pathogens, as the level of internalization varied greatly with crop type and within same crop, probably because studies were done with much higher concentrations of pathogens than would naturally be present in the field (Wachtel et al., 2002; Mitra et al., 2009; Zhang et al., 2009).

In view of all the above, persistence of *Salmonella* in the soils and plants can place burdens on lower end of the food chain for reassuring the safety of fresh produce, particularly those often consumed raw. As the survival of enteric pathogens remains to be not easily explained, rather affected by complex interactive factors, evaluating risks at the retails level is important to assist in the development of risk mitigation strategies.

1.3. The responsibility of food service businesses in fresh produce safety

1.3.1. Implications of food service in fresh produce-related food outbreaks

In principle, everyone in the food chain has some degree of responsibility for food safety. The current industrial treatments of fresh produce did not completely remove pathogens (Goodburn & Wallace, 2013), hence the lack of an efficient kill-step pose challenges on the fresh produce industry to enhance preventive measures, such GAP and hazard analysis of critical control points (HACCP). Food service establishments (FSE) such as restaurants, hotels, bars, and cafeterias are considered an important source of foodborne outbreaks (Olsen et al., 2000) and have been largely incriminated in several food poisoning outbreaks involving fresh produce (CDC, 1999, 2007; Naimi et al., 2003; Calvert et al., 2007; De Jong et al., 2007; MacDonald et al., 2011; Nicolay et al., 2011; Smith et al., 2011).

In England and Wales, the food service sector is considered to be the common source for reported foodborne disease (Gormley et al., 2011; Mody et al., 2011). Nearly 85% of all outbreaks occurred as result of food mishandling in FSEs or homes (Hall, 1997). In the US, restaurants were implicated in 61% of the identified outbreaks of foodborne illness from 2004 to 2008 (CDC, 2011). In Canada, the Canadian Public Health Agency reported on 30 people ill in the *E. coli* 0157:H7 outbreak associated with shredded lettuce served at fast food restaurants. Whereas two of the largest multi-state outbreak of STEC O157:H7 infections of 2012 were also linked to consumption of romaine lettuce. (Slayton et al., 2013). According to available data, produce-related foodborne illnesses had a great likelihood to have taken place during preparation by infected food workers (Hall, 2004) and in varying degrees among countries in Europe (Calvert et al., 2007; Gutierrez Garitano et al., 2011; Nicolay et al., 2011; Smith et al., 2011). In one of the largest recent outbreak, more than 140 people were infected by *Salmonella*-contaminated domestic tomatoes served during the 2002 in US Transplant Games at Disney's Wide World of Sports Complex in Florida (CDC, 2002). Additionally, several restaurant *E. coli* O157:H7 outbreaks in Oregon and Washington in 1993 were associated with a variety of items from the salad bar. All the restaurants obtained their beef from the same source, and it was the practice to trim, macerate, and marinate the beef in the same kitchens used for preparation of fruits and vegetables for the salad bar. It appeared that that some raw beef was the source of contamination for the fresh produce (Doyle et al., 2006).

In Australia, lettuce in salad mix was implicated in a food outbreak of *Salmonella* Bovismorbificans where deficiency in equipment cleaning was reported as possible cause (Stafford et al., 2002).

To date, 189 food poisoning cases and 240 cases of dysentery were registered in Lebanon, further to a food poisoning incidence in September 2014 that affected 40 people admitted to hospital, with the highest peak of reported cases in the summer. The majority of the reported cases in Lebanon have been linked to consumption of meat. A study by Harakeh et al. (2005) showed that *Salmonella* and pathogenic *E. coli* were detected in meat- based fast food containing vegetables and sesame seeds oil**.**

Therefore, inadequate sanitization and poor handling of fresh produce at subsequent stages towards the end users, such as during preparation and storage, may intensify the risks of microbial contamination, particularly when raw vegetables are not subjected to further processing for elimination of microbial contamination (Coleman et al., 2013), which in this case emphasize the enactment of safe handling practices (McCabe-Sellers & Beattie, 2004).

1.3.2. Contributing risk factors to foodborne illnesses

Data on risk factors indicate that most incidences resulted from improper food handling practices, contaminated supplies, dirty food contact surfaces, poor personnel hygiene practices (Clayton et al., 2002b; Green et al., 2007). Other factors included inappropriate storage temperatures, and insufficient cooking (Kaferstein, 2003; WHO, 2007; Jones et al., 2008), and unsafe food source (CDC, 1996; Kaferstein, 2003).

A review of Gormley et al. (2011) on food outbreak cases for the period 1991-2008 identified again that the contributory factors in most outbreaks in England and Wales being cross-contamination, inadequate heat treatment, and inappropriate food storage.

Several studies showed that the main sources of cross-contamination during processing originate from food contact surfaces, equipment and employees (Gill & Jones, 2002).

Employees with limited knowledge and poor personal hygiene have a major role in the prevention of foodborne diseases since they may cross-contaminate raw and ready-to-eat food (RTE) as asymptomatic carriers of food poisoning microorganisms (Walker et al., 2003a; Kimura et al., 2005; Todd et al., 2008).

In a review on a collective data on food outbreaks in US, Canada, and European countries including Australia, Greig et al. (2007) indicated that hand contacts of food handlers during preparation of food are probably the common reason of food handlers' implication in most cases. It is widely recognized that inadequate hand washing and use of gloves for extended periods of time are the most common mechanism resulting in cross-contamination of RTE foods (Green et al., 2006).

Equipment and surfaces can become vectors of pathogens in the event of inadequate cleaning (Evans et al., 2004) and in the lack of hygiene awareness (Audit commission.1990*)* as low level and absence of training was shown to be directly correlated with poor hygiene. Almost half of the reported cases of foodborne outbreaks in France in 1998 were related to contamination by equipment with biofilms (Haeghebaert et al., 2001). Non-sanitized and scratched cutting surfaces, combined in some cases with misuse of sanitizers, are probably an effective environment for harbouring pathogens that have the propensity to form biofilm resisting washing applications (Pui et al., 2011). The use of plastic cutting boards have gained popularity in the last two decades with the introduction of plastic cutting board in the 1970s in replacement of wooden cutting board for reducing the risk of cross-contamination mostly from remaining juices of raw meat and poultry on the surface resulting in the transfer of microorganisms to other foods on the same surface (Gough & Dodd, 1998). Nevertheless, it was also shown that cutting boards act as vehicles of pathogens to food (Cliver, 2006) and that domestic food contact surfaces still represent a critical risk of cross-contamination and recontamination events (Redmond & Griffith,2003).

A number of citations focused on survival and transfer of pathogens including *S.*Typhimurium from food of animal origins to surfaces or other food types in meat preparation (Gough & Dodd, 1998; Kusumaningrum et al., 2004; Moore et al., 2007; Ravishankar et al., 2010) and different factors influencing the attachment capacity of *Salmonella* spp. were suggested; for instance, Pui et al. (2011) indicated that attachment is strongly strain dependent, others pointed out that the rates of transfer of S*almonella* cells between various types of surfaces can be affected by type of bacteria and moisture levels on food surface or type of contact surfaces (Milling et al., 2005), inoculum size (Montville & Schaffner, 2003) and conditions of the source and destination (Sattar et al., 2001; Gill & Jones, 2002; Goh et al., 2014).

Therefore, in conditions of hygiene failures in restaurants or home settings, remaining pathogens can attach to food preparation surfaces in the food processing environment (Zottola & Sasahara, 1994; Joseph et al., 2001; Bae et al., 2012) and capable of forming biofilms which shall potentially act as a continuous source of post-processing bacterial contamination and pose significant health hazards (Stepanović et al., 2004). Heavily chipped cutting boards and crevices are ideal surfaces to harbour pathogens that are capable of forming a biofilm resisting to disinfection and sanitization and this resistance can be due to the failure of the sanitizer or disinfectant to penetrate the biofilm, the development of bacterial stress response and the quorum sensing within the matrix (Mah & O'Toole, 2001). Different types of cutting boards (glass, plastic, wood, stainless steel) were examined to understand conditions that enhance bacterial attachment and its transfer after sanitization (Soares et al., 2012b). Additionally, the transfer rate of pathogens from cutting surfaces to sliced food was determined in several studies (Ravishankar et al., 2010; Zilelidou et al., 2015). Ravishankar et al. (2010) concluded that contamination risks would still occur after washing in view of the limited reduction in transfer rate from cutting board and knife to lettuce (45.62%), whereas by using soap and hand-hot water the population of *S*. Newport on lettuce slice was reduced by less than 1 log. Similarly, when hands were washed with soap and hot water (48ºC), *S*. Enteritidis cells were still isolated from contaminated surfaces (Humphrey et al., 1994). On the contrary, a significant reduction in cross-contamination of *Campylobacter jejuni* was reported when higher water temperature (68ºC) was used to wash the cutting boards for 10 s along with soap and brushing (De Jong et al., 2008).

1.3.3. Barriers to compliance with safe food handling practices

Food service employees often perceived time constraints, funding for training, and lack of resources (i.e., financial and time) as barriers to safe handling (Taylor, 2011), in addition to employees' motivation (Sneed et al., 2004). Other studies indicated space as impediment to compliance with standards and food safety systems (Yapp & Fairman, 2006; Howells et al., 2008).

Clayton (2002a) investigated food handlers' beliefs and self-reported practices in 52 small and medium sized enterprises (SME) in Wales. Lack of time, understaffing, limited space and resources were again major hurdles for enactment of safe practices. In addition, food handlers' perception of the risk of implicating foodborne illness in their business was recorded low which indicated the significance of risk-based approach in developing training. In a more recent study, Howells et al. (2008) investigated the perceived barriers in the context of compliance to proper handwashing practices, thermometer use, and cleaning practices of contact surfaces. In this study the data collection took place in focus groups: ten groups had untrained staff in food safety (n = 34) and twenty focus groups had staff who received ServSafe training (n = 125). For groups, limited time, inconvenience, lack of resources and training were main barriers to hand washing, thermometer use and cleaning of work surfaces. Whereas the inconvenient location of sinks and drying of skin deterred staff from proper hand washing. Similarly, Green et al. (2007) noted that hand washing was more likely to occur in restaurants that provided food safety training, with more than one hand sink, and with a hand sink in the worker's sight.

Motivations, management involvement, monitoring activities and training, staff turnover, salaries have been shown to affect food handlers' behaviours and attitudes in applying learnt knowledge (Ehiri & Morris, 1996; Walker et al., 2003b; Seaman & Eves, 2010).

To reduce foodborne illnesses, hazard and risk-based quality management systems are essential. However, SMEs tend to have limited adoption of HACCP (Fielding et al., 2005). Often the food management systems are perceived as complicated (Bas et al., 2006) and burdens with insufficient guidance and access to support (Taylor & Kane, 2005). In addition, the barriers against the implementation of food management systems, mainly in SMEs, were reported to be due to a lack of understanding of HACCP and hygiene knowledge of food handlers (Walker et al., 2003a).

1.4. Research approach in evaluation of determinants of food handlers' behaviour

1.4.1. Knowledge, Attitudes and Practices of food handlers

Researchers recognized that knowledge in food safety is essential for safe food handling practices (Ehiri & Morris, 1996a; Fielding et al., 2005). In view of its essential role, mandatory or voluntary training mandates for food handlers was adopted (Egan et al., 2007) considering trained and knowledgeable staff in food safety tend to be more aware and handle food safely (Angelillo et al., 2000). The assessment of knowledge and its influence on practices is often based on the Knowledge, Attitudes and Practices (KAP) model that have been addressed over the last three decades in different European and American countries

(Manning, 1994; Lynch et al., 2003; Bermudez-Millan et al., 2004; Bolton et al., 2008; Jevšnik et al., 2008), in Asia (Rao et al., 2007), and only to a limited extent the Middle East, in Jordan (Osaili et al., 2013). Many studies investigated KAP of food handlers as an intervention to study post-training effects and to improve food safety knowledge and behaviours. The KAP model denotes that an individual's behaviour depends on knowledge level, which when enhanced, it directly affects individual's attitude and practice (Rennie, 1995).

Often a great majority of KAP studies used questionnaires that comprised self-reported practices and were either delivered for completion or completed via interview with food handlers. The results of various works varied, although limited knowledge on contamination, cross-contamination and temperature control were often common (Jevšnik et al., 2008; Buccheri et al., 2010; Osaili et al., 2013). In Slovenia, food safety knowledge and practices of food handlers working in food businesses (n=386) were assessed using self-administered questionnaire. Most of the respondents showed limited knowledge on microbial hazards, and temperature control (Jevšnik et al., 2008). Similarly, In Turkey, Burcu Tokuç (2009) investigated knowledge, attitudes, and practices of food handlers in hospitals with questionnaires administered through face-to-face interview. Authors reported that less than half of food handlers (41%) lacked the knowledge on temperature control of food and refrigerator temperature (27%). They also observed that food handlers' self-reported practices were not correlated with their attitudes.

Likewise, food handlers working in food businesses in Turkey (n=764) demonstrated limited knowledge in food safety particularly in areas related to temperature control. However, trained staff scored higher than untrained, whereas the scores on attitudes were also reported in this study to be generally higher than self-reported practices (Bas et al., 2006).

In a study on knowledge, attitudes, and practices of food service staff (n=502) in nursing homes and long-term care facilities, Buccheri et al. (2010) observed that knowledge on temperature control and high risk foods was limited despite that the majority of respondents (82%) were trained in food hygiene. It was also evident that self-reported behaviour pertaining to temperature and cross contamination control was poor when attitude to food hygiene was generally positive.

Jianu and Chiş (2012) conducted a cross-sectional study on food handlers working in Romanian SMEs. The knowledge was significantly higher in food handlers with higher educational levels and those in higher position levels compared to their counterparts. However, trained food handlers did not demonstrate high knowledge, which indicated the need for retraining of food handlers. Whereas, the score on knowledge did not significantly differ in relation to gender, age, or professional experience, but experience levels were major elements that affected knowledge level. Hislop and Shaw (2009) also reported that food handlers in the food service industry scored less than 50% on the food safety knowledge. The cut off score was set to 70%, as the minimum required by the health authorities in Edmonton for certification. Non-certified scored between two to five times less than certified food handlers and those with over 10 years of experience scored the least indicating that training had a positive impact on food safety knowledge but refreshing of information is important for knowledge retention.

Santos et al. (2008) used interview based questionnaires to investigate the KAP of food handlers (n=124) in 32 school canteens. Results showed no relationship between food handlers knowledge and self-reported behaviour ($r = 0.09$, $p > 0.05$) indicating that participants probably tend to report intended or correct practices instead of actual ones. Trained staff scored significantly higher than untrained staff, however, in general, food handlers' practices were affected by the peak working hours ($X_2 = 13.9$, $p < 0.001$) and education levels was significantly associated with safe practices ($X_2 = 10.7$, $p < 0.01$).

A study in Jordan reported 69.4% total score of correct answers, with trained food handlers scoring significantly higher than untrained, 62.5 ± 21.7 and 52.2 ± 19.6, respectively (Osaili et al., 2013). In his comprehensive review on KAP studies, Egan et al. (2007) also noted that whereas most authors measured effectiveness of training on knowledge which varied among studies, generally, the improved positive attitude toward food safety and knowledge was not supported by self-reported practice, and he touched on the discrepancy between self-reported practices and the actual behaviour.

In summary, studies relying on food handlers' self-reported practices may not reflect accurate assessment in view of inherited bias in the responses when respondents are more likely to project desired behaviours than the actual (Egan et al., 2007; Green et al., 2005). Researchers demonstrated that food handlers who received training were more knowledgeable in food safety and tend to adopt safer behaviour than untrained food handlers (Angelillo et al., 2001), while others asserted that the training of food handlers does not necessarily guarantee safe food handling practices and that several factors other than knowledge, education, and training influence safe food handling behaviours (Clayton et al., 2002a).

Therefore, in evaluating food handlers' practices, direct observation is recommended by WHO as the most reliable method for measuring compliance to hand hygiene (Boyce et al., 2002). The inspection of food handling premises and processes are also important public health tools designed to reduce incidents of food-borne illness backed up with public policy enforcement, education, and communication of risk by publicizing inspection scores (Seiver & Hatfield, 2000).

Surprisingly, there has not been sufficient observational studies conducted since the applications of the tool in research in the late 80's (Powell & Attwell, 1995; Tebbutt &

Southwell, 1989; Wyatt & Guy, 1980). This is probably because observation studies require intensive time and human resources, although its use provides more reliable data on food handling practices as employees may overestimate their actual behaviours bias (Clayton & Griffith, 2004).

1.4.2. Observation assessment tool

Researchers used observational tools to obtain actual measurements of changes in practices subsequent to introduction of food safety programs. Mitchell et al. (2007) suggested more research work to be based on data of actual food handlers' practices. Observations are reliable tools to capture actual practices as employees tend to overestimate their actual behaviours, thereby introducing social desirability bias (Clayton & Griffith, 2004). They also provide an effective measurement of food safety culture-supporting intervention material (Powell et al., 2011).

Scientists reinstated this tool in their research approach for its high relevance to verify reported practices and gain in depth understanding of impediments against implementation of HACCP in catering companies (Garayoa et al., 2011) or evaluate hand washing and cleaning of utensils and implements practices in assessment of trainings (Soares et al., 2012a). For example, in the US, Strohbehn et al. (2011) conducted an observation study of food handlers' practices in 16 food service operations in Iowa, US. The authors identified practices that greatly contribute to cross-contamination risk; these were related to deficit sanitization and lack of cleaning standard operating procedures. Other researchers revealed that food handlers were engaged in improper practices related to cooling and thawing of foods, lack of documentation and operating procedures, i.e., food workers did not record refrigerator and freezer temperatures (Sneed, Strohbehn, & Gilmore, 2004). Similarly, Henroid and Sneed (2004) reported improper food cooling and thawing, lack of food temperatures monitoring, and infrequent handwashing in 40 food service operations in Iowa state.

Strohbehn et al. (2008) conducted a five three hours' observation periods of employee (n=80) hand washing behaviours during menu production, service, and cleaning in 16 food service operations for a total of 240 h of direct observation. The sample comprised food operations in assisted living, schools, childcare centres, and restaurants. Overall, authors noted that the hand washing practices were not frequent as per Food Code requirements, neither according to recommended methods. Almost all employees did not wash hands between handling raw and handling ready-to-eat food. Frequency of compliance to proper handwashing was 31% and 33% in childcare and assisted living, respectively, and lower among food staff in school who washed hands 22% of the recommended times.

An observational assessment to study food staff behaviours (n=33) working in deli foods in nine stores was conducted to determine the level of compliance with the Food Code by using a notational analysis protocol focusing on hand washing and the cleaning of equipment, utensils and surface (Lubran et al., 2010). Authors reported low compliance rate in hand washing practices and that 67% (295 of 439 observations) of the actions for which hand washing was recommended at the chain stores and 86% (235 of 273) of those at the independent stores were noted for employees touching non-food contact surfaces prior to handling RTE food

Notational analysis was employed earlier by Clayton and Griffith (2004) who recorded the observations of 115 trained/certified food handlers in 29 catering establishments in Wales during hand hygiene practices, cleaning of food contact surfaces and equipment, washing of utensils, and use of different utensils for preparing raw and RTE foods. Each food handler was observed on three separate occasions performing over 270 actions. The study showed that appropriate hand hygiene practice in 31% of the required occasions, however this was not observed after touching potentially contaminated surfaces, after touching hair and face, and after handling potentially contaminated food. Authors identified fundamental hand hygiene errors represented by failure to use soap and dry hands. Moreover, third of the observed staff (31%) cleaned food contact surfaces adequately in 33% of the time, whereas a substantially higher frequency of adequate washing of utensils and appropriate use of different utensils were in 91% of occasions observed. The authors conclusively demonstrated that the training based on knowledge delivery and certification was not effective for promoting safer hand hygiene and work surfaces cleaning practices, rather more effective methodologies should be adopted to transfer learning into practice.

According to Seaman and Eves (2008), knowledge play an essential role in the enhancement of behaviours and practices, however, it is not the only factor that would lead to proper food handling unless complemented with other factors. The provision of effective food hygiene training and the effective application of safe food handling practices acquired through training programs are vital to controlling food-borne illnesses throughout the world; yet, training alone is not proven the only variable correlated with safe and proper practices in food premises. The results of the latter study concur with a number of studies which demonstrated that employees have sufficient knowledge about safe food handling; however, several improper food handling practices have been still identified (Henroid & Sneed, 2004; Strohbehn et al., 2008).

Henroid and Sneed (2004) evaluated food handling practices, presence of prerequisite food safety programs, and employees' food safety knowledge and attitudes in 40 Iowa school food service operations to determine readiness for implementing HACCP programs in school food service operations. These researchers demonstrated that employees had high food safety knowledge, 15.9 ± 2.4 over 20 points and overall positive food safety attitudes, ranging from 4.2 to 4.8 over 5 points. However, observations of food handling practices indicated that proper food handling practices sometimes were not followed.

The number of studies that reported inadequate food safety practices despite high levels in food safety knowledge is concerning when handling RTE raw vegetables. There is very little information on food handling practices pertaining to the preparation of fresh vegetables in restaurants. Interestingly, Coleman et al. (2013) recently showed a satisfactory trend in handling leafy greens in the majority of premises he examined, pointing out at the significant influence of training on safe handling of leafy greens and the records for traceability in accordance with the FDA guidelines almost overall locations. In his study, Cenci-Goga et al. (2005) examined 894 samples in a university restaurant, before and after implementation of the HACCP system and personnel training. Microbiological indicators levels and incidence rates of pathogens were reduced. The microbial results of this study demonstrated that personnel training together with HACCP application contributed to improve the food safety of foods.

In summary, the review of observational studies always pointed at the limited role of training in enhancement of practices, while cited barriers were typically the responsibility of businesses being responsible for supporting appropriate and safe daily operations in order to alleviate perceived barriers.

1.4.3. Social cognitive theory

As multiple factors contributing to the success of food safety practices remain unexplained, continuous research efforts directed toward the improvement of food safety practices in food services. Yiannas (2009) emphasized the importance of considering behavioural theories and looking at the various aspects that can influence behaviour within an organization, of which, the applications of the TPB gained attention in a number of studies in recent times (Hinsz & Nickell, 2007; Clayton & Griffith, 2008; Ball et al., 2010).

TPB is a social cognition theory introduced by Ajzen in 1985. It emerged as a framework to understand and predict changes in human behaviour (Ajzen, 1991). According to the TPB, an individual's behaviour is determined by behavioural intentions, which are influenced by attitudes, and social norms, i.e., subjective norms, and perceived behavioural control. Individual's intention to embrace a particular behaviour will theoretically be enhanced with increased positive attitude toward their ability to perform a behaviour and positive feedback from important others (Ajzen, 2006). Increasing intention to change and control over a particular behaviour increases the likelihood of adoption of a behaviour (Figure 1.4) (Ajzen, 2011).

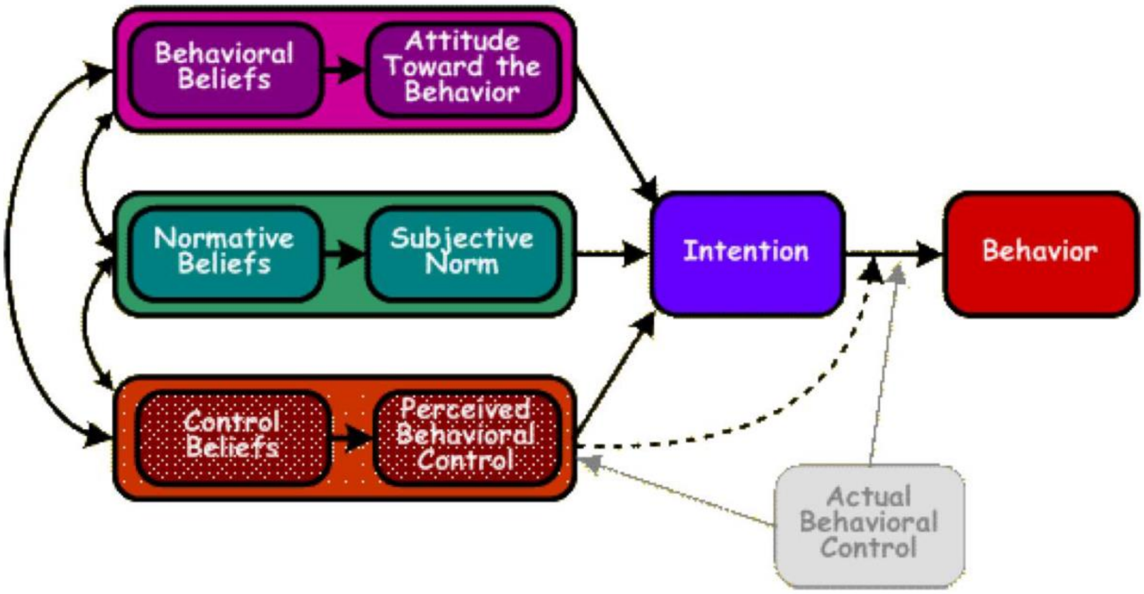


Figure 1.4. The components of the TPB model (Ajzen, 2006)

Perceived behavioural control is determined by personal beliefs about how difficult or easy it is to perform the behaviour. Subjective norm is an individual's perception of whether important others in their social and work sphere think that the behaviour should be performed. If perceived behavioural control is a determinant of food handlers' behaviour, in this case, the understanding of factors that hamper or enable behavioural control is instrumental in developing intervention.

Studies that used the TBP revealed further attribute of training effect on food handlers' behaviours. Training was shown to significantly influence the subjective norms of food handlers as trained food handlers expressed concerns on what others thought of their behaviour in the workplace (Seaman & Eves, 2008). Using questionnaires based on the TPB, interviews with food handlers (n=155 food handlers, n=10 managers) in nurseries, day care centres, preschools, respite units, and residential homes showed that neither training nor attitude had a significant influence on the intentions to perform safe food handling practices on all occasions. However, subjective norms (the opinions of important others) significantly affected food handlers' behavioural intention to perform safe food handling practices ($\beta = 0.55$, $p \leq 0.001$). The instrument was also useful to explain that managerial training in food safety and their in-house training and support for food handlers may reduce the risk of foodborne disease outbreaks as more untrained food handlers expressed positive intentions for training. The managerial role and influence of management factors were determined by other researchers (Clayton & Griffith, 2008). They applied the social cognitive theory to examine factors influencing hand hygiene practices of 115 food handlers during food preparation and hygiene actions (n = 31, 050). The Hand Hygiene Instrument (HHI) was developed to measure participants' attitudes toward hand hygiene practices which was based on the TPB framework. Multiple regression analysis showed that the TPB explained 34% of the variance in hand hygiene malpractices; a large fraction the variance remains unexplained which means other factors influencing behaviour change within the individuals and within the environment are still to be explained. In general, the results revealed that attitudes (β= -0.20), subjective norms (β= 0.20), descriptive norms (β= 0.23), perceived behavioural control β= -0.47) and intention (s = -0.20) were significant factors that explain and affect hand hygiene malpractices. The food safety practices of supervisory staff and co-workers had influence on food handlers' intentions to perform hand hygiene actions. Consequently, the authors emphasized on the important role of organizations or business in any food safety interventions.

Arendt and Sneed (2008) conducted a study to determine factors that motivate employees to perform tasks of cleaning and sanitizing, hand washing, wearing clean uniforms, and recording temperatures. A sample of 169 students surveyed in three hospitality management classes at a Midwest university using open-ended questionnaires. The overall responses were coded thematically into motivation factors related to, 1) policy and standards, 2) accountability, 3) role model, 4) training, 5) reward and punishment, and 6) resources.

This study provided additional confirmation on the primary responsibility of businesses in food safety activities as those themes were factors that are tightly pertinent to the supervisory role and responsibility to lead by example in enactment of safe practices. The authors maintained that the training of supervisors is an important requisite for the motivating staff to enactment of safe food handling practices and supervisory role should be considered in designing a culture of food safety culture. In their earlier work, Clayton et al. (2002a) stated that food safety practices will only be implemented given adequate resources and an appropriate management culture.

1.4.4. Organization food safety culture

The role of organizational culture in changing employee behaviour has been originally studied in areas related to occupational health and safety to understand the organizations through a cultural framework with a focus on values, attitudes and beliefs of members (Flin, 2007; Guldenmund, 2007; Piers et al., 2009).

The attitude, behaviour, and commitment of the leaders in the organization are regarded as major indicators of an existing safety culture. Reason (1995) asserted that culture evolves progressively as affected by conditions, past events, the character of leadership, and the dynamics of the workforce. Therefore, food safety problems in the food industry are partly caused by behavioural issues, including those involving organizational culture (Griffith et al., 2010b; Taylor, 2011). As several factors in an organization were reported to influence staff food safety practices, e.g., time, resources, including those related to organizational culture, there has been a growing research interest in understanding the role of organization factors in changing food safety behaviours (Mitchell et al., 2007; Pragle et al., 2007). The aspects of organizational culture vary in the context of different types of industries, however, the major co-existing ones are, management/supervision, risk–taking, application of safety systems, and pressures of work environment pressure which include work space (Flin, 2007; Guldenmund, 2007). The management bears a large share of responsibility to develop and communicate food safety mission statement, to allocate budgets for food safety and to demonstrate consciousness in targeting a change in behaviour and setting up a food safety culture (Powell et al., 2011). Communications and sharing information within the business environment and among co-workers about foodborne risks are major attributes in an organization culture and greatly contribute to a culture of food safety (Yiannas et al., 2009).

The food safety culture is one facet of organization culture, a concept that has recently emerged. In recognition of the importance of safety culture in improving workers' safety behaviours in occupational safety and health fields, researchers opted for its assimilation into a workable concept in the food service industry. It is evident that food safety culture is an emerging risk of foodborne illness outbreaks in food service organizations (Griffith et al., 2010b) in view of striking food outbreaks in retails that were largely incriminating the business itself (Powell et al., 2011).

The concept of food safety culture was introduced to understand how organizations do food safety, however there is no definite consensus on its definition (Griffith et al., 2010a) and on the best approach to measure the culture elements needed to cultivate safe food handling practices or the role of organizational culture in actual food safety performance

(Clarke, 2000). Researchers adapted measurements used in different fields, while some tools involved assessment and measurements of elements that were primarily based on expert opinions. Some of the organizational cultural elements pertinent to occupational safety and health research were adopted as basic constituents of food safety culture (Griffith et al., 2010a). The latter was assessed through employees' perceptions toward the management system and style, leadership, communication, sharing of knowledge and information, accountability, risk perception, and work environment. Taylor (2011) viewed food safety culture as influenced by 20 interconnected elements related to knowledge (awareness, technical expertise, training), attitude/psychological (agreement, risk awareness, self-efficacy, motivation), external (inspection, government/industry guideline), and behavioural (organizational culture, resources, competence).

Chapman et al. (2010) introduced training intervention using communication tools presented by food safety information sheets (posters) to support a good food safety culture. The authors employed video observation of food handlers' practices as they are exposed to changing information posted in their working environments. Food handlers (n=47) were observed for hand washing and cross-contamination prevention practices. When food safety information sheets were posted and changed each week for a period of 7 weeks, the intervention contributed to significant improvement in all the occasions observed. Hand washing attempts increased significantly by 6.7% ($t = -4.482$, $p < 0.001$) and correct hand washing outcomes by 68.9% %($t = -2.253$, $p = 0.029$). Furthermore, significant reduction of the indirect cross-contamination events by 19.6 ($t = 2.939$, $p = 0.005$) were observed. While there was an improvement, Chapman (2010) noted that risky behaviours still existed in these establishments. Hence, the risk of food-borne disease transmission via food workers can be effectively reduced if other methods (theory-based training and organizational change) are used along with interventions.

More recently, Neal et al. (2012) used the food safety climate tool developed by Ball et al. (2010) to evaluate the food safety culture in restaurants. Management commitment and food safety behaviour of staff were the two major factors. Employees' perceptions of food safety culture were compared based on their demographics. No significant differences were observed in perceptions of food safety culture among restaurant employees with different years of food service experience, time worked at the present job, prior food safety training, and food safety certification. Frash and MacLaurin (2010) reported that employees' perceptions of organizational culture were significantly influenced by job positions and that considering the heterogeneity of culture within an organization, the food safety culture should be assessed across those subcultures. Similarly, the relationship between employees' perceptions of organizational culture and employees' attitude and intention were different between those with and without food safety certification (Lee et al., 2012b).

Earlier research by Ball et al. (2010) was undertaken using the Food Safety Climate tool to assess key factors that may influence the compliance of meat processing plant workers to food safety procedures. The authors developed the tool of 65 elements measuring five workplace factors: management commitment, work unit commitment, food safety training, infrastructure, and worker food safety behaviours, which were classified into five factors by factor analysis.

Employees' perceptions toward management system and style are inspired and guided by the "coordinated activities that direct or control food safety," management involvement in day to day operations and practices. Leadership is evaluated by measuring the extent to which staffs are influenced by their leader(s), to perform and comply with business food safety standards. Communication is assessed by the quality of top-down, and bottom-up shared messages, also by the exchange of food safety information among co-workers. The environmental factors include tangible factors such, e.g., availability and accessibility of hand wash basins or other hygiene equipment, and adequate staffing to ensure performance of safety practices as intended (Clayton et al., 2002a). Organizational support is also an element in employees' perception of environment support (Clayton et al., 2002a) that is assessed via varied indicators related to rewards, roles, job satisfaction, empowerment and responsibilities (Griffith et al, 2010a).

In summary, the understanding of the food safety culture provides insights into factors and reasons for non-compliances to safe food handling practices at work. Efforts to assess and establish positive food safety culture and to better define its role in improving food safety practices can be facilitated by its measurement. Various definitions of safety culture exit and these included attitudes and beliefs about several factors in an organization culture which often referred to as food safety climate (Griffith et al., 2010a).

Elements and tools varied among studies; there were factors that were constantly measured and those include the management support and commitment, system and process, e.g., procedures, communication, and resources, and employee attitude and behaviours). While at the same time, overlapping components may be encountered. Management systems and management involvement in daily operation activities are assumed to be a major and most frequently relevant component. Those are supposedly catalysts of the in-house communication process essential for the continuous monitoring and improvement activities, management review and trend analysis.

1.5. Washing methods of fresh vegetables in SMEs

The use of sanitizing agents for produce washing has been recommended particularly for ready to eat fresh-cut produce that are not subjected to further cooking or processing in order to ensure the safety of fresh produce (Ruiz-Cruz et al., 2007). Washing is one of the most important method for reducing fruits and vegetables contamination, by removing soils, insects, chemical products and some microorganisms from the surface of fresh produce (Ruiz-Cruz et al.,2007).

Plant surfaces are complex in nature and characterized by differences in their morphology and metabolic processes of the leaves, stems, and roots of vegetables which provide the pathogens suitable environments for survival. For instance, the convoluted nature of a parsley or lettuce leaf serves as a protective harbour for pathogens making it difficult for sanitizers or water to penetrate. However, in the event of loss in the integrity of the surface, e.g., bruises or cuts, bacterial growth can be rapid (NACMCF, 1999); this and the contamination risks along the food chain pose further challenges on food service sector for elimination of pathogenic risks.

The persistence of pathogens on vegetables in various conditions and stages of the food chain is well recognized (Islam et al., 2004; Kisluk et al., 2012) and can place burdens on lower end of the food chain for reassuring the safety of fresh produce, particularly of those consumed raw. Under certain conditions of storage, growth may occur especially on fresh-cut leafy greens. The nutrients inside the plant become available and promote multiplication to hazardous levels (FDA/CFSAN, 2001) and cell attachment is enhanced due to hydrophilic bruised and cut surfaces (Ells & Truelstrup Hansen, 2006).

Several studies investigated different sanitizing agents such as chlorine and new agents such as chlorine dioxide, ozone, peroxyacetic acid, electrolyzed water, and organic acids on reducing different levels of inoculated pathogens on the surface of whole and fresh-cut vegetables at different storage and treatment temperature (Karapinar & Gonul, 1992; Escudero et al., 1999; Akbas & Olmez, 2007; Ölmez & Akbas, 2009). The review showed that a major fraction of those studies used different methods and concentration levels of sanitizers which made comparisons not easy.

1.5.1. Effect of chlorine

The effectiveness of chlorine and chlorine-based derivatives in disinfecting water has been well known for over 30 years (Gomez-Lopez et al., 2008). The numerous studies that investigated the effect of chlorine and chlorine-related sanitizers on fresh leafy vegetables did not generally differ in their results. While the reductions levels were greater compared to using water alone, the disinfection effect of variety of chlorine-based sanitizers at permissible levels were often limited to 1–2 log units reduction of pathogenic populations on the surface of produce at a treatment level of 50-220 ppm for 1 to 2 min and even up to 10 min. contact time (FDA, 2015c). It is thought that they are the most commonly used sanitization agents throughout the industry, with free chlorine concentrations of 50–100 ppm (Gil et al., 2009). However, their effects were not particularly significant on lettuce. For instance, *L. innocua* levels were reduced by 1-1.5 log CFU/g when shredded lettuce was washed agitated for 5 min in 10 ppm sodium hypochlorite (NaClO) (Francis & O'Beirne, 2002); nonetheless, the applications of higher concentrations of NaClO (100 ppm) for 1 min. did not improve the sanitization effect and reduced *L. monocytogenes* by only 0.7 log CFU/g. Temperature of treatment and longer wash times did not increase the elimination of microorganisms which was also demonstrated to vary among types of pathogens (Francis & O'Beirne, 2002). Similarly , Li et al., (2001) showed that the treatment of lettuce by submersion for 1 min in 20 ppm NaClO at 50ºC and 20ºC reduced *E.coli* O157: H7 by 1.1 log CF/g and 1.0 log CFU/g. Similar reduction levels in *L. monocytogenes* (1-1.2 log CFU/g) were reported by (Li et al., 2002) when lettuce was submerged in only 20 ppm NaClO for 30 s. Scientists applied similar concentrations (100 ppm) for longer time, 10 min., on cabbage, lettuce and parsley, the results showed log reductions of *E. coli* by 1.41, 0.72 and 1.56 log CFU/g, respectively (Seymour et al., 2002).

Interestingly, higher reductions were observed when lettuce samples were dipped for only 2 and 5 min in the same concentration of the sanitizers (100ppm NaClO), reductions in the *E. coli* and *L. monocytogenes* levels recorded 2.6-2.9 and 1.5-1.7, respectively (Akbas et al., 2007).

Zhang and Farber (1996) showed that the population of *L. monocytogenes* inoculated onto shredded lettuce and cabbage were reduced by 1.7 and 1.2 log CFU/g, respectively when treated with 200 ppm chlorine for 10 min. As exposure time was increased from 1 to 10 min, the log reductions slightly increased. However, the earlier study by Beuchat et al. (1999) showed no significant difference in the effectiveness of 200 ppm chlorine and treatment with deionized water on removal of *E. coli* O157:H7 when the exposure time increased from 1 to 5 min.

Gram negative pathogens were more resilient at higher concentrations. Washing lettuce with agitation for 1 m. in 200 ppm NaClO reduced *E. coli* 157:H7 and *Salmonella* levels by only 0.86-0.88 and 0.96-1.04 log CFU/g, respectively (Koseki et al., 2003b). The elimination in populations of *Shigella sonnei* was substantially higher (7 log CFU/g) on parsley leaves after treatment for 5 min with 250 ppm free chlorine (Wu et al., 2000). Likewise, earlier work by Wei (1995) indicated that *Salmonella* Montevideo inoculated into the cracks of mature green tomatoes survived treatment with 100 ppm chlorine.

Yersinia enterocolitica showed a high vulnerability to chlorine compared to reported results on other pathogens. The treatment with 100 and 300 ppm chlorine for 10 min. contact time resulted in population reductions of approximately 2-3 log on shredded lettuce, which have not differed greatly with temperature rise of the solution from 4 °C to 22 °C. Combination of treatments was also studied to improve the efficacy of chlorine (Escudero et al., 1999). The addition of 0.5% lactic acid to 100 ppm chlorine inactivated *Yersinia.*

enterocolitica by >6 log. The additional of a surfactant, Tween 80, to hypochlorite led to reduction in microbial numbers by 99.6%, but this has resulted in organoleptic changes.

It is maintained that the failure of conventional water and chlorine based sanitizers to remove more of the pathogens is ascribed to the neutralization of some of chlorine before coming into contact with microbial cells as it reacts with organic matter and exudates from tissues of cut produce surfaces, thereby reducing its effectiveness, in addition to the survival of microorganisms in protective hydrophobic pockets or crevices, cracks, and small fissures on the produce surface (Parish et al., 2006). Hence, applications of detergents and surfactants coupled with physical manipulation, i.e., brushing may be used to reduce hydrophobicity or eliminate part of the wax, enhance accessibility of sanitizers to microorganisms,

As commercially available alternatives to chlorine are limited in their ability to kill bacteria attached to produce surfaces, more effective sanitizers are needed in order to exceed the reduction of 1–2 log units. The higher chlorine concentration (2000ppm) for 1 min resulted in a maximum reduction in human pathogens of 2.3 log CFU/cm^2 on apples, tomatoes and lettuce dipped in chlorine concentration (2000 ppm) for 1 min (Beuchat, 1998) and < 90% reduction of several strains of *Salmonellae* inoculated onto fresh-cut cantaloupe cubes (Beuchat & Ryu, 1997). Therefore, it is generally agreed that higher level of chlorine may not necessarily eliminate the microorganisms and may actually cause loss of satisfactory organoleptic characteristics. There are concerns on the repercussions of the continuous use and in some case misuse of chlorine in the disinfection process on environment and health for the formation of carcinogenic halogenated disinfection by-products (Ölmez & Kretzschmar, 2009), and in some cases the practice may be against national standards or regulations. (Parish, 2006). Most of the current investigations have been focused on the search for alternative sanitizers such as organic acids based on assuring the quality and safety of the produce.

1.5.2. Organic acids

The organic acids such as acetic acid and citric acid, and vinegar (acetic acid) have been examined in various studies for their effectiveness in removing pathogens from fresh fruits and vegetables (Karapinar & Gonul, 1992; Rhee, 2003). Most pathogenic organisms do not grow at low pH (<4.5) and the antimicrobial components of organic acids are fully protonated which can penetrate the bacterial cell membranes (Bjornsdottir et al., 2006). Other mechanisms of antimicrobial property of organic acids are attributed to pH, acid concentration, and inhibition of enzymatic activities, disruption of membrane transport and metabolic processes (Blackburn & McClure, 2002).

The results of many studies also varied, considering the various factors that alter the antimicrobial activity of organic acids, and these include pH, acid concentration, bacterial strains and environment (Bjornsdottir et al., 2006).

The potential use of acids or in a combination with chlorine and lemon as a simple sanitizing method in the food service sector was examined. Additionally, vinegar and lemon juice have been demonstrated as inexpensive, simple household sanitizers, although changes in sensory effects when used in high concentration on produce would be a drawback to it use. In further details, Wu et al. (2000) showed that treatment with vinegar solution of 7.6% acetic acid for 5 min at 21 °C or 250 ppm free chlorine reduced the populations of *S. sonnei* on the on whole parsley leaves by more than 7 log CFU/g (to undetectable levels <0.6 log CFU/g), which surpassed the effect of chlorine. Treatment with 5.0% (v/v) acetic acid or 200 ppm free chlorine also effectively reduced the microorganism population by more than 6 log CFU/g. However, changes in the colour and strong vinegar odour were noted with 2.6% acetic.

The effect of acetic acid on elimination of pathogenic bacteria on fresh parsley was studied by Karapinar and Gonul (1992). Dipping parsley leaves in 2% acetic acid or 40% vinegar for 15 min has reduced *Yersinia. enterocolitica* by >7 log cycles. However, the antimicrobial activity of acetic acid was not equally effective on other pathogens. Dipping apple inoculated with *E. coli* O157:H7 in 5% acetic acid for 2 min. at room temperature reduced the pathogen populations by more than 3 log CFU/cm as compared to less than 3 log by 80 ppm peroxyacetic acid (Wright et al., 2000).

In addition, the storage time and temperature after treatment or sanitization has been considered in similar studies. For example, Chang and Fang (2007) examined the antimicrobial effect of rice vinegar containing 5%, 0.05% and 0.5% (v/v) acetic acid on the survival *E. coli* O157:H7 and *S.* Typhimurium inoculated on shredded iceberg lettuce. Samples were stored at 4 C for 14 days and 22°C for 7 days to determine the growth and survival of pathogens. Populations of both pathogens were reduced by 1 log CFU/g at the end 4°C post-treatment storage, However, microbial levels on shredded lettuce increased 3 logs within 3 days at 22 C. Earlier, it was demonstrated that pathogens can survive and resume growing after washing produce. The populations of *E. coli* O157:H7 showed an increase in levels on lettuce and cucumbers during storage at 21ºC (Abdul-Raouf et al., 1993).

At a lower concentration of acetic acid (0.5%) mixed with mustard, the growth or survival of *E. coli* O157:H7 and *L. monocytogenes* were limited. The authors concluded that the antagonistic effects may be changed if mustard is used alone or in combination with >1% acetic acid (Rhee et al., 2003).

Treatment with lemon juice was more effective in eliminating viable *S*. typhimurium cells on carrots than treatment with vinegar (Sengun & Karapinar, 2004). The mixture of lemon juice and vinegar (1:1) resulted in populations reduction of 5.64 log CFU/g and low 2.58 log CFU/g at high and low inoculum levels, respectively, to an undetectable level after 30 min treatment; whereas treatment with lemon juice and vinegar alone for different exposure times (0, 15, 30 and 60 min.) resulted in a significant reduction ranging from 0.79 to 3.95 and 1.57 to 3.58 log CFU/g, respectively. An earlier study demonstrated the bacteriostatic action of vinegar containing 0.1% concentration of acetic acid on *E. coli* O157:H7 and its enhancement due to the synergistic effect of additional sodium chloride (Entani et al., 1998).

Lactic acid used alone or in combination with other chemicals, has been shown to be effective in eliminating *E. coli* and *L. monocytogenes* on iceberg lettuce (Akbas & Olmez, 2007). Inoculated lettuce was dipped 0.5% citric acid or 0.5% lactic acid solutions or chlorine solutions for 2 and 5 min. The number of *L. monocytogenes* and *E. coli* were reduced with chlorine solution by 1.0 and 2.0 log CFU/g, respectively, while higher reduction (2.0 log CFU /g) was achieved by lactic or citric acids for in *E. coli*, and about 1.5 log CFU/g with lactic acid for *L. monocytogenes*. The authors concluded that dipping of iceberg lettuce in 0.5% citric acid or 0.5% lactic acid solution for 2 min could be an effective alternative to chlorine for reducing microbial populations on fresh-cut iceberg lettuce. The latter study concurred with earlier work of Zhang and Farber (1996) that showed chlorine treatment not to differ greatly from lactic and acetic acid (0.5, 0.75, 1%); it was equally effective to lactic acid at 1% in reducing *L. monocytogenes* levels on shredded lettuce. Chlorine 100 ppm in combination with lactic acid and acetic acid (0·75 or 1·0%) was more effective in reducing levels of *L. monocytogenes* than either lactic acid or chlorine alone. In addition, lactic acid was more effective than acetic acid in reducing numbers of *L. monocytogenes*, although with maximum reductions of 0.5 and 0.2 log, respectively, after a 10 min exposure to 1% solutions of each organic acid.

Treatment with citric acid in the form of lemon juice has been also shown to reduce populations of *S* Typhimurium inoculated onto cubes of papaya and jicama from 4.9 to 2.3 log CFU/g when examined after 6 h storage post- treatment (Fernandez Escartin et al., 1989). Treatment of cubes of watermelon and papaya inoculated with *Campylobacter jejuni* with lemon at room temperature, the reduction in the populations ranged from 0 to 14.3% of the original inoculum compared to 7.7 to 61.8% in fruits without lemon juice added. In general, the effectiveness of lemon juice was dependent on types of produce, showing higher antimicrobial activity on papaya.

In summary, the bactericidal effect of organic acids was notably observed on *Yersinia enterocolitica* (Escudero et al., 1999; Karapinar & Gonul, 1992). Treatment and storage temperatures of 4°C and 22°C were common in several studies (Zhang & Farber, 1996; Escudero et al., 1999; Akbas & Olmez, 2007; Chang & Fang, 2007). The washing procedures occurred before the establishment of the protective extracellular polysaccharide, yet the washing process does not remove all the bacteria (Zhang & Farber, 1996). Overall, the efficacy of the different washing solutions did not surpass the standard reduction of 2 log in bacterial contamination while retaining satisfactory organoleptic characteristics.

1.6. Problem statements, research gaps and thesis Rationale

This research is a multidisciplinary work that investigated multi-dimensional areas and related factors in approaching food safety from farm-to-fork. For this, problem statements, research gaps and thesis rationale are presented thematically in this section with reference to the literature review.

1.6.1. Fresh produce sector in Lebanon

Despite some improvements made to legislations and to the quality of specific agri-food products, substantial work is still required to reassure a safe local agri-food production due to difficulties in implementing adequate food safety control measures. The latter are currently performed by several ministries because of ineffective coordination and multiple inspections by various departments using different guidelines (Abou Ghaida et al., 2014). This hypothetically fosters a number of unsafe practices, which they reportedly escape accountability (El-Jardali et al., 2012). In the implementation of such systems, accountability is a fundamental axe in a multidimensional system of a food safety culture (Griffith et al., 2010a) and it is of high relevance for a food safety culture at macro level (governmental level), particularly in this part of the world. For instance, in the EU, as Künast and Schmidt put it “Food safety issues can have huge political implications” drawing their statement from the emergence of BSE which led to major political and structural changes in UK as well as in Germany where both the agriculture and health ministers resigned and a reform movement was formed towards more consumer protection oriented ministries (Federal Government of Germany, 2001). Moreover, the establishment of EFSA in the year of 2002 could be a valid example as to the consumer oriented culture and regulations geared to reinforce the public trust (EFSA, 2012). Along the same lines, the new rules required by USFDA established mandatory safety standards for produce farms and make importers and food processors more accountable for reducing foodborne illnesses by verifying that imported food meets US safety standards.

Farm size, farmer’s education, and poverty are often reported as major drivers for the non-compliance to standards in the developing countries (FAO, 2014; World Bank, 2008). However, the constraints that vary among those countries, i.e., the food safety governance and institutional settings and enabling environment have not been so far researched in the context of the food safety reassurance of local fresh produce. Additionally, there is a need for information that is relevant to those involved in promoting reforms and institutional innovations. Lebanon can be considered as an exemplary model of many countries in the MENA region in view of resemblances in constraints, e.g., mismanagement, overlapping responsibilities, inconsistency with international standards, lack of regulations and effective enforcement, lack of capacity, inadequate surveillance mechanism, poor infrastructure and institutional fragmentation (FAO, 2012b, 2014).

Given this background, the current food safety system in Lebanon hypothetically predisposes the fresh produce production to food safety challenges as affected by lack of accountability, poor support needed to improve the quality and safety of fresh produce in the local market; hence the local consumers' food quality and health protection is supposedly marginalized.

Furthermore, there has not been any work, regionally and in Lebanon, that addressed the food safety issue of the fresh produce chain and underlying risk factors by a combination of observational and microbiological tools along the fresh produce chain. The effective application of a national food safety system in Lebanon needs to be built based on the current food safety issues and data on the prevalence of microbiological hazards that are potentially of significance. As such, an analysis of the current state of food safety governance in Lebanon and the regulatory system will critically identify root causes for the ineffective control of fresh produce safety destined for local consumption. At the same time, the mapping of the fresh produce supply chain shall contribute to the literature available and reveal the critical points and practices that manifest high microbial populations and pose health risks to consumers.

1.6.2. Food service sector

SMEs constitute a great proportion of enterprises (90%) currently active in the economy and trade in Lebanon (Naimy, 2011; MoE, 2014) and are hypothetically the weakest node in the chain being limited in their food safety performance in view of various constraints to take adequate sanitary and preventive measures (Taylor & Kane, 2005). The implementation of food safety practices is burdensome for SMEs (Yapp & Fairman, 2006) which reportedly lag behind larger businesses in terms of their compliance.

In this context, the earlier report by UNIDO (2002) on meetings with stakeholders to evaluate the Lebanese food law and regulations and existing food inspection activities revealed a number of shortcoming in the SME's implementation of safety measures in the food preparation (Box 1.1).

Box 1.1. A list of reported limitations for reassurance of food safety (UNIDO, 2002)

- Laws are far from being complete or up to date
- Lack of co-ordination among different government bodies
- No scheduled control of quality and safety of food products is undertaken for local products nor for imported products
- Many production units are supplying the market without any control
- Standards for food products are being developed
- Safety rules and technical guidelines for food products including GHP, GMP and HACCP are starting to gain momentum
- Lacks modern technology and equipment
- Evident lack of education at all levels
- Absence of a rapid effective surveillance system
- Lack of food safety practices in a large number of food factories
- Lack of a credible, responsive regulatory system

To date, there are no published articles or reported information on the food safety performance of the food service sector neither in Lebanon nor in other countries in the region. Additionally, the evaluation of food handlers' awareness on food safety and identification of their attitudes and perceived barriers have not yet been studied; hence, the potential impact of lack of engagement in safe practices is concerning, especially in relation to handling RTE salads vegetables. On the other hand, as observational studies often focused on handwashing practices and on food handling practices of RTE and foods of animal origins, a significant knowledge gap still exists on handling practices of RTE salads vegetables in SMEs and their association to microbial quality. Therefore, an in-depth understanding of food handlers' knowledge, the common handling practices of RTE salads vegetables and limitations to safe practices are essentials to put in place food safety interventions and reduce bacterial hazards.

However, the application of TPB and KAP models and assessment of food safety culture via measurement scales of organizational culture elements are developed in more developed countries and may not necessarily apply in developing countries due to the diverse social and cultural backgrounds. These models are often based on individual worker's perception of an organization's culture, hence the limitations in their applications as employees' perceptions of organizational culture are greatly influenced by their job positions (Frash & MacLaurin, 2010) and hypothetically by their knowledge. Food workers with limited knowledge in food safety are likely to reflect inaccurately on their own practices, on their perceptions of management performance and commitment to food safety. Consequently, this may lead researchers to inaccurate interpretations and depiction of an image that contradicts the reality.

Therefore, considering the heterogeneity of culture within an organization, it is theoretically more valid to found an opinion or judgement on factors affecting safe food handling practices through direct valuation of food safety values at the high level of the food system hierarchy, i.e., direct observation of management influence, not solely through food workers. The rationale of this present study corroborates with a very recent study where authors selectively chose staff with experience and knowledge in the subject to examine onsite food safety culture (Fatimah et al., 2013; Fatimah et al., 2014)

The management system is one indicator of an organizational food safety culture, among others, i.e., risk perception, leadership, communication, and environment (Griffith et al. (2010a), which distinguish companies with food safety values. The lack of management commitment has been reported as a barrier to implementation of management system (Macheka et al., 2013), hence the significance of the latter in evaluating organizational food safety culture (Ball et al., 2010; Neal et al., 2012).

SMEs in Lebanon are run by families (Naimy, 2011) or by entrepreneurs. In other cases, SMEs are well known outlets of local corporates that are specialized in the hospitality industry and that are well received in the market for its branded operations locally and/or internationally. It is thus assumed that the latter are considered to be adequate in resources, skills and probably committed to serve safer food in the market.

In this respect, it is more common that individual organisations or corporates consider the effective food safety management as an essential element in their organisational strategy in endeavours to maintain stakeholders trust and to protect corporate brands or reputation already attained by investment and resources inputs (Manning, 2007). Hence, FSEs managed by corporates are more likely to apply systems and provide appropriate conditions for safe practices on premises (Manning, 2007) which reflects management awareness and perception of risks; therefore, staff would be hypothetically more responsive to comply with food safety requirements when strictly enforced by a proactive management. On the contrary, small family- or entrepreneur-owned businesses are relatively managed by the owners or a couple of persons in charge. The lack of a formal management structure and communication in theory repeal concerns for trends analysis and improvements in food safety.

Given this background and considering the potential biases from self-reported practices and responses that may be based on incorrect assumptions by respondents, commonly with training shortcomings, it is important to determine elements that support safe practices and a food safety culture by employing a combined tool, i.e. KAP model combined with visual assessments of food safety climates, in studying the variations of variables, i.e., food safety climate and food handling practices in FSEs operated by two distinct types of management.

1.6.3. Fresh vegetables: washing methods and handling practices in SMEs

Researchers have examined various sanitizers for their efficiency on reducing pathogens attached on produce surface (Karapinar & Gonul, 1992; Wu et al., 2000; Rhee et al., 2003; Abadias et al., 2011). Most of these results were generally reported on studies conducted either in the framework of washing processes applied in the whole and fresh-cut industry or based on laboratory settings. They employed different methodologies, applications and preparation of treatments, strains, inoculum levels, attachment time of bacteria on produce, washing methods and produce varieties (WHO/FAO, 2008b); very few studied parsley. Parsley is widely used in the Lebanese cuisine for a wide array of cold appetizers and traditional salads, and is prepared in large volume. In this case, there is still uncertainty about the actual influence of the local washing methods, storage and temperature conditions together with culinary practices typically applied in this region on reducing bacterial contamination on parsley.

In a different context of food preparation, it has been widely demonstrated that cutting boards can act as vehicles of pathogenic microorganism to foods (Pui et al., 2011). The review of literature showed that studies concentrated on the transfer rate of pathogenic microorganisms from cutting boards to a single sliced food, or from food of animal origin to cutting boards (Ravishankar et al., 2010; Pui et al., 2011). However, available studies did not investigate the pathogens transmissions from surfaces to vegetables, mainly parsley, when several batches of vegetables are consecutively chopped in scenarios that mimic the actual practices in SMEs. The preparation of parsley in large quantities, at home or in the restaurants, is typical in Lebanon and the Middle East. It is hypothesized that cross-contamination rate to parsley will be reduced over the chopping process and further lessen on washed cutting surfaces. The quantification of pathogen transmission to parsley leaves should be considered and shall provide valuable information on the risk of cross-contamination associated with parsley preparation.

1.7. Research aims and objectives

The broad aim of this research is to identify the range of factors that influence the microbial safety of fresh produce from farm-to-fork in Lebanon through approaching the sector regulatory and different FSEs' management structure.

In pursuit of the main aim, this research employed qualitative and quantitative forms of analysis to attain several objectives.

The specific objectives are:

- To analyze the institutional and regulatory framework and determine areas of strength and weaknesses in the administration of the fresh produce sector and local market.
- To identify bacterial hazards, agricultural practices and critical areas that are most likely to contribute to the risk of bacterial contamination of fresh produce along the farm-to-table continuum in Lebanon.
- To evaluate the food safety knowledge of food handlers in SMEs, their attitudes and food handling practices in different management environment.
- To determine the key elements for a food safety culture in the SMEs and the contribution of management types in reducing barriers and risk factors.
- To investigate the relationship between the food handler's practices and food safety climates and the microbiological quality of fresh salads vegetables.
- To evaluate the efficacy of different washing and sanitation practices commonly applied in the SMEs in the reduction of *Salmonella* on fresh parsley to determine optimum conditions and applicable solutions.

- To determine the trend of cross-contamination and the extent of *Salmonella* transmission during the chopping of parsley based on observed practices.

1.8. Description of the research plan and thesis outline

1.8.1. Research plan

In order to meet the objectives of this research, the research plan was based on a holistic approach. Hence, it is set into 4 phases (Figure **1.5**).

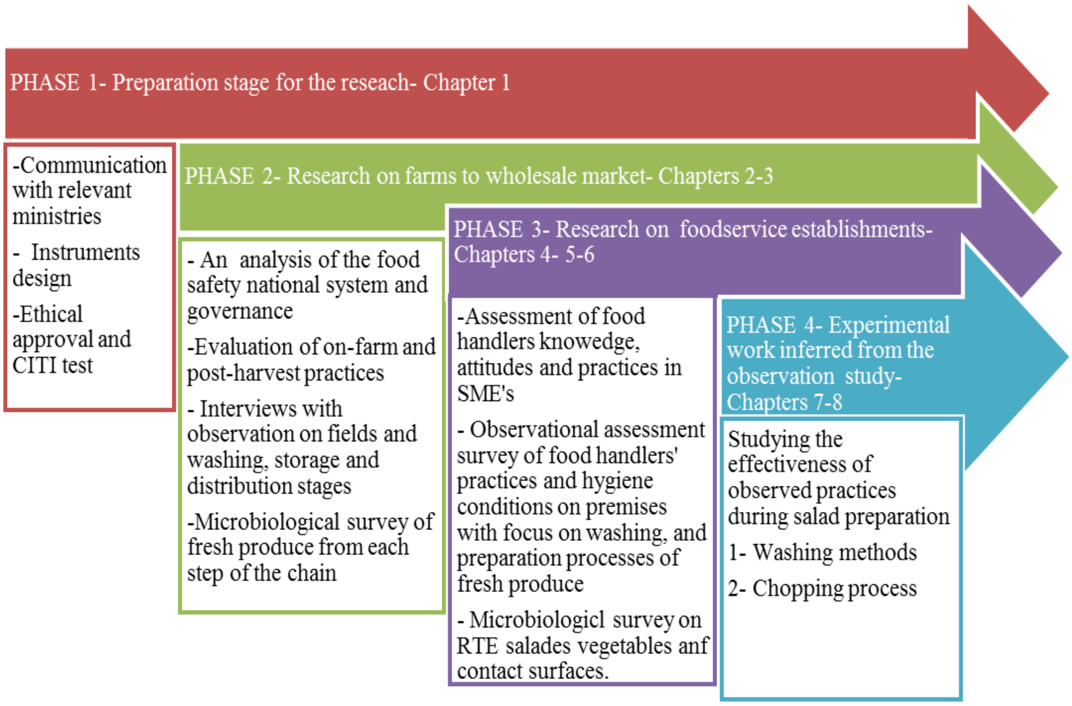


Figure 1.5. The four phases of the thesis research

Phase 1 is described in this section, whereas a detailed description of the successive phases (2, 3 and 4) is presented in details in the materials and methods of the relevant chapters.

The phase 1 preparation stage involved communication with relevant ministries to access data on licensed businesses and in attempt for collaboration.

Before the implementation of the research project, meetings were arranged with representatives in the Ministry of Economy (MoE), MoPH, Ministry of Tourism (MoT) and the municipality of Beirut (all involved in the food safety) to obtain a list of food businesses in Beirut, and get further clarification on the defined role of each department. Several limitations were encountered (Box 1.2).

Box 1.2. Limitations Encountered During Communication Stage

- **Collaboration**

The initial plan for collaboration with the department of consumer protection in the Ministry of Economy in running the survey at a large scale was not realized in view of the ethical consideration of the research not to disclose names on enterprises and on data property.

- **Accessibility to Data**

Restaurants: The public sources for data on locations and number of operating restaurants were not available in all relevant ministries, except with the Ministry of Tourism that indicated many food businesses are operating without licenses or get their one-year temporary license from the ministry of tourism and don't proceed with renewal. The list obtained from the syndicate of restaurants contained few restaurants located in Beirut itself.

Farms: An official letter was submitted to the Minister of Agriculture in request for information on registered farms. The ministry considered that the information bear sensitive and private data, and may trigger farmers' resentment. The request was rejected.

Faculty of Agriculture – American University of Beirut: Attempts to seek academic staff support was not successful due to limited information.

Limitation in data is common as well to other sectors such as the water sector, and this is due to information-hoarding by institutes and the slow recovery of monitoring agencies from the various impacts of the civil war (Farajalla et al., 2015). According to Mhanna (2016), this is also due to absence of archiving data at project completion which make information accessiblity to successors impossible.

Research instruments described in relevant chapters were also developed and prepared in this phase. The protocol and questionnaires were constructed to meet standards required for conducting research involving the participation of human. The researcher- (myself) was trained and certified by the Collaborative Institutional Training Initiative (CITI). The research assistant for the on-farm assessment survey, fulfilled the same requirements set by the American University of Beirut before assisting in this part.

The Internal Review Board of Plymouth University (Faculty of Science and the Environment, Research Ethics Committee) reviewed the tools and approved the protocol prior to administration.

All ethical considerations were met and have been approved by Plymouth University and the American University of Beirut for conducting research.

1.8.2 Thesis outline

This thesis is organized into nine chapters.

- Chapter 1 presents the introduction to the thesis which includes detailed literature reviews relevant to the topic of each chapter. It also identifies the strengths and weaknesses of the methodological approaches taken to investigate the research questions in the context of FSE, problem statements and gaps, aims and objectives, research plan and thesis outline.
- Chapter 2 presents an in-depth analysis of the general condition of the local agricultural sector, constraints and incentives. It also presents the specific areas whereby the institutional and regulatory framework of food safety and the political decisions are linked to and interfere on the course of food safety governance.
- Chapter 3 presents empirical data on agricultural practices and environment from farm to wholesale markets and results on the routes of contamination of fresh produce. The trend

in the microbiological contamination of fresh produce across the different stages of production were also described.

- Chapter 4 studied the knowledge, attitudes and self-reported practices in food safety of food handlers working in FSEs operated by different types of management.
- Chapter 5 investigated further the adequacy of food handling practices including fresh vegetables preparations by using observational assessment tool. In this chapter, observed practices were assessed in relation to self-reported practices and to management types.
- Chapter 6 described the microbiological quality of fresh salads vegetables, food safety environment and handling practices in SMEs to determine a link between microbial hazards on vegetables and the associated risk factors and unsafe food handling practices.
- Chapter 7 studied the effect of the decontamination effect of the washing solutions on *S.* Typhimurium contaminated parsley. In this chapter, washing methods and handling conditions identified during the observation survey were adopted. Control measures and recommendations were highlighted.
- Chapter 8 addressed quantitatively the risk of cross-contamination and the transfer of pathogens from cutting boards to parsley during the chopping process under conditions resembling those typically occurring in SMEs.
- Chapter 9 summarizes the findings, research limitations and highlighted a number of areas where future research needs is proposed.

2. The role of inequity and political incoherence as primary risk factors for food safety - a focus on the fresh produce chain

2.1. Introduction

"Traditional farmers tend to be among the least educated and oldest segments of the population" (World Bank, 2008). In addition to education, the farm size is reported as a factor that affect the ability of farmers to comply with food safety standards (FAO/WHO, 2005b; FAO, 2014). In Lebanon, the reassurance of the food safety is also influenced by inadequate capacity development, monitoring and surveillance mechanisms, and the lack of a risk-based food safety law (Abou Ghaida et al., 2014). Nonetheless, there are numbers of initiatives, national projects and programmes that aimed to support the agricultural sector and proved to be successful through the improvement of the Lebanese agricultural products and linkages with the tourism sector (MoA, 2014). At present, there is still limited information on the specific areas whereby the institutional environment in Lebanon impacts the safety of the local fresh produce chain. In order to evaluate and strategize solutions for fresh produce safety, it is important to examine the nature of local national policies and priorities, and to formulate an effective strategy based on evaluating the socio-political structure. The objective of this chapter is to analyse the regulatory and institutional framework and identify the strengths and weaknesses in the context of fresh produce.

2.2. Materials and methods

An in-depth qualitative analysis was undertaken by employing a desk review as it was complex to conduct any statistical techniques due to scarcity of published scientific data. This method served to develop a tentative rudimentary conceptual framework into its final form in chapter 9. The inputs were related to technical knowledge, research background and personal experience which was supported with microbiological and observation surveys of this research work.

The desk review covered

- Scientific papers
- Statistical information
- Recent reports of regional and national workshops or meetings
- Relevant materials, i.e., the evaluation of the current agricultural strategy of the Ministry of Agriculture (2010-2014) and (2015-2019), documents of the FAO report “Lebanon Country Programming Framework, 2012-2015, the report on the state of the environment “State and Trends of the Lebanese Environment” and latest reported Agricultural Census 2010.
- Related reports by local and international experts.

2.3. Overview on the agri-food sector

According to the most recent report published in 2012 on the Agricultural Census (Census 2010, 2012), Lebanon’s agriculture accounts for an average of 6.4 percent of Gross Domestic Product (GDP) (FAO, 2012b). The total agricultural land area is estimated at 332,000 hectares, of which 231,000 hectares are cultivated. The cultivated area reached 232,200 hectares in 2010, which included seasonal, protected and permanent crops.

Land use in Lebanon has gradually shifted from production systems based on cereals towards more intensive production (mainly fruit and vegetables) resulting in a higher level of agricultural added value per hectare of agricultural land. The seasonal cultivable lands were estimated to be 102,470 hectares of which 36% is used for growing vegetables. The latter comprised leafy greens and other vegetables in 18% and 42% of cultivated lands, respectively (Census 2010, 2012).

The country is divided into 40 agricultural homogenous zones with very distinct socio-economic and geopolitical characteristics. The zones located in the Bekaa and northern Lebanon provinces cover 67 percent of the total agricultural land and typically belong to large commercial farmers, whereas the South is characterized by small farms mostly in rural areas (MoA, 2014). Nearly 71% of the cultivated land is directly exploited by 84% of the license holders (Figure 2.1). Whereas lands that are used in an indirect way such as in return for money (i.e. rent, leasing or production services) amounted to 48,596 hectares which represent 21% of the total agricultural area.

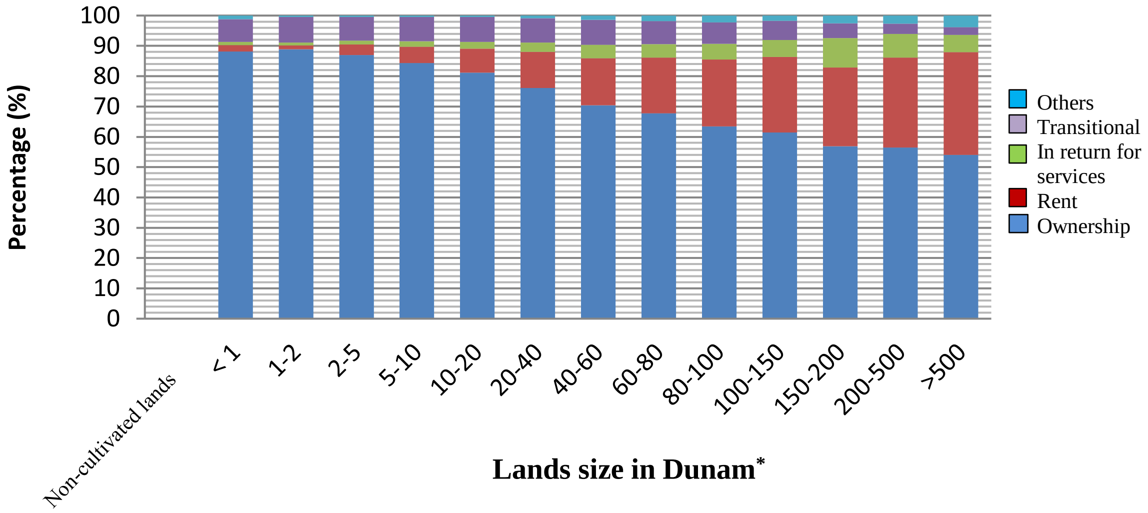


Figure 2.1. Lands exploitation, by size of holding area (dunam) (Census 2010, 2012)
*1 dunam= 0.1 hectare

The most fertile areas are located along the coastal strip and in the Bekaa Valley. Agricultural production is concentrated in the Bekaa, which accounts for 42 percent of total cultivated land (MoE, 1991).

Seasonal planting distributed by province shows that Bekaa predominantly accounted for the cultivated area (31%), followed by 29% for Baalbek-Hermel and Akkar (20%), while 2 and 5% were recorded in the rest of the provinces.

Table 2.1. Distribution of agricultural products (hectare) for the year 2009 by province

Product	Nabatieh		South		Bekaa		North		Mount Lebanon		**Total**
	Area	%	Area	%	Area	%	Area	%	Area	%	
Cereals	3895	7	2782	5	41178	74	7234	13	556	1	**55646**
Legumes	1437	23	562	9	2236	36	1562	26	339	6	**6136**
Vegetables	768	2	1918	5	23396	61	10739	28	1534	4	**38355**
Fruits trees	2985	4	13434	18	29853	40	17165	23	11195	15	**74632**
Olives	9656	17	10224	18	3408	6	27832	49	5680	10	**56800**
Other trees	171	3	1443	22	580	9	214	3	4177	63	**6585**
Other agri-products	312	6	260	5	4108	79	312	6	208	4	**5200**

Adapted from MoA (2016)

A volume of 44% (7,299.6 hectares) of leafy vegetables are cultivated in the Bekaa region which refers to the Middle and West Bekaa excluding North Bekaa (Baalbeck and Hermel) (Figure 2.2). Lettuce cultivation amounted to 2,591.8 hectares (36% of leafy vegetables cultivation) and is mostly located in the Bekaa region that produces 61% of the total lettuce cultivation in Lebanon (MoA Census, 2010).

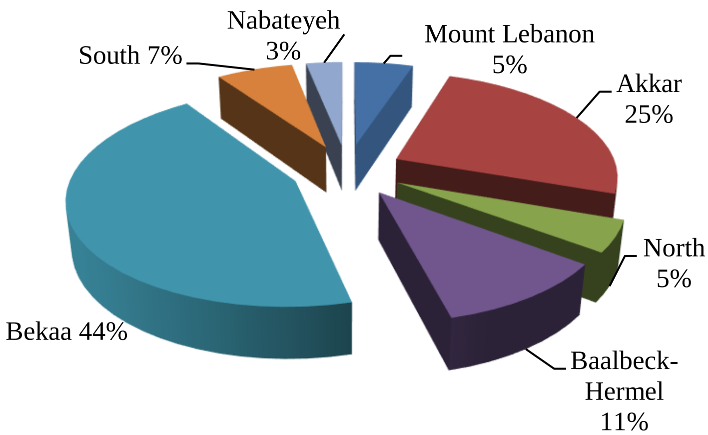


Figure 2.2. Leafy vegetables production by province
(MoA 2014; Census 2010, 2012).

2.4. Social and economic characteristics of the agriculture sector

According to the report of the Ministry of Agriculture (MoA), the agricultural sector in Lebanon employs 6 % of the total labour force. A total of 170,000 farmers or farm holders have an average age of 52 years, half of which depends solely on agriculture for their livelihoods (MoA, 2014). Overall, poor salaries and the lack of social security make the agricultural sector unattractive for young people despite of a high percentage of young people (45% below the age of 24) and an elevated rate of unemployment amongst the youngest (22.6 %). The Lebanese rural population that is involved in the agriculture sector reached 817,000 people in 2010 with an average household size of five persons per household. However, only 11% of the licensed growers' benefits from the Agricultural Extension Service, 70% of these beneficiaries receive such service from an agricultural engineer and 7% from veterinarian (Census 2010, 2012).

Lebanon has a fertile land, rich natural resources and a Mediterranean climate suitable for varieties of seasonal fruit and vegetable production (Michaels et al., 2010). Furthermore, the strategic location between Europe and the Arab Gulf states offers Lebanon a distinct advantage for the export of high-value fresh and processed horticultural crops. However, Lebanon is a net importer of food and agricultural products and its dependence on agricultural imports is further exacerbated by the Syrian refugees hosted in the country (FAO, 2012a). The domestic production fulfils merely 20 percent of its domestic requirements. The gap is covered by imports. The value of imports reached a total of LBP 5,173,678 million (3.45 billion USD) in 2013 in comparison to a total value of exports LBP 1,141,994 million (0.76 billion USD).

Nevertheless, Lebanon's comparative advantage in agriculture production lies in the fruit and vegetable sector which is supposed to place less strain on its water resources than cereals or livestock production (Michaels et al., 2010; CIHEAM/IAMM, 2014).

According to the report issued by the Centre International des Hautes Etudes Agronomiques Méditerranéennes, one of four Mediterranean agronomic institutes of the International Centre for Advanced Mediterranean Agronomic Studies, the main agricultural exports are fruits and vegetables (37%) of which about 39% are exported to Arab Gulf countries, including Saudi Arabia (17%), United Arab Emirates (10%), Kuwait (8%) and Qatar (5%), plus Jordan (9%) and Syria (12%) (CIHEAM/IAMM, 2014). As for the EU, Lebanon was ranked as the 43rd in the exports from the EU and 112th in imports to the EU. In 2004, agricultural products were among exporting items destined to the EU, which constituted one fifth (19.3%) of all Lebanese exports (Markou & Stavri, 2005). The EU is Lebanon's main trade partner and the Association Agreement signed with the EU in 2002 stems from an overall European strategy towards securing Euro-Mediterranean partnership in economic development, security matters and social and cultural affairs. Despite the Association Agreement, exports to the EU are less than imports, with less than 10% of total agricultural exports in 2013. To access international markets, improvements in the agriculture sector should be addressed via upgrading and enhancement the safety and quality of its primary products and by reducing costs.

2.5. Food safety dimensions and influence of politics

2.5.1. Food safety law

Food safety is a recent concept, not only in Lebanon, rather in the Arab world and is increasingly deemed as an essential public health issue in the Arab region. Some countries, like the UAE and Jordan, supported by the WHO undertook enormous efforts to review their existing food safety systems and update their national legislation. However, other countries including Lebanon are still struggling to meet the international standards and to enact a modern food safety law according to local needs and legal mandates (CSPI, 2005). As a result of persistent problems, including mismanagement, overlapping responsibilities and lack of communications among the different government bodies (FAO/WHO, 2005a; El-Jardali et al., 2014), the process of updating regulations, effective enforcement, capacity building and adequate surveillance mechanisms is slow (FAO/WHO, 2005a; FAO, 2012a; Farajalla et al., 2015). Consequently, there is currently still no comprehensive national legal framework to govern the food safety from farm-to-fork. The course of food safety governance seems to be hampered by the political structure (Markou & Stavri, 2005) and conflicts in ministerial roles and disagreements within the cabinet on developing an independent Lebanese Food Safety Agency (LFSA). This means, that also the changes proposed with the new draft and the regulation framework (risk assessment and risk management) will be dependent on political decisions (Abou Ghaida et al., 2014; Organization of Consumer Protection, n.d.). It is obvious that the political instability in Lebanon led further to a weakening of its institutional capacities (UNIDO, 2015), and provides fewer opportunities to further the reform process. The improvements in the effectiveness of the food safety systems in other countries in the region as a result of a substantial improvement in the food safety governance (FAO/WHO, 2005b) are examples of the successes possible with a supportive environment in relatively stable countries. For instance, Jordan, classified as a high-middle income country like Lebanon (UNIDO, 2015), has established the Jordan Food and Drug Administration (JFDA) which has the authority to inspect food products at the retail and wholesale distribution levels (CSPI, 2005; FAO/WHO, 2005b). At the same time, the UAE, a high income MENA country, moved towards modern risk-based approaches to food safety management. It adopted the use of customized software for food inspection to monitor and control the safety of domestic and imported goods (FAO/WHO, 2005b).

2.5.2. Roles and responsibilities of government bodies in the national food control system

Currently, the food safety system structure in Lebanon is still fragmented and the food safety law and legislations are underdeveloped to match modern requirements and emerging food hazards (FAO/WHO, 2005a ; El-Jardali et al., 2014; Farajalla et al., 2015) (Figure 2.3).

The nutrition and food safety in Lebanon is an issue dealt with by several ministries: MoPH, MoA, MoT, MoE, Ministry of Industry (MoI) and the Ministry of Labor (MoL) (El-Jardali (El-Jardali et al., 2014).

The regulations of food safety and hygiene fall under the umbrella of the MoPH through the Health Sanitation Department, the Nutrition Unit and the Central Public Health Laboratory (Appendix A). The role of the MoPH in food control is limited to sampling and reporting results to juridical authorities, in case of detected threats and risks. MoT issues the license of business operation to FSEs and hospitality industry sectors based on classification scheme (Stars classification) that incorporate requirements pertaining to structural conditions, such as parking lots, number of toilets, laundry facilities, handwashing washing sinks, availability of a food safety system. A sample example of the criteria is presented in Appendix B (Criteria for restaurants classification-in Arabic). In this page, the criteria indicate mandatory fulfilment of sanitary conditions, lighting and availability of electrical generators for all the classification levels. It also indicates that a restaurant with HACCP or ISO 22000 system will be classified according to number of stars obtained plus “S” (i.e., “excellent status”). Whereas, the municipality is more or less directly responsible in ensuring that licensed businesses (in the context of food sector) satisfy the sanitary conditions though relevant inspections.

The responsibility of the MoA is confined to fresh produce, local meat and animal feeds and the slaughterhouse. It is the designated institution for the formulation of the agricultural sector strategic framework and the development of related policies and programmes (MoA, 2014). This includes the development of the legislative and regulatory frameworks governing the agricultural sector, and securing infrastructure to facilitate investment, production and marketing operations. In addition, it has a primary role in the management of natural resources (agricultural land, irrigation water, forests and forestry, fisheries, rangelands) and in the preparation and implementation of rural development programme (MoA, 2014).

The MoE is formally involved in setting standards and specifications, but also widely recognized for its activities in inspections of FSEs through the department of consumer protection (MoE, n.d.); besides MoPH, and local municipalities. At the industry level, inspection process is undertaken by MoI and MoL. The coordination among all those ministries was reported to be weak already earlier (UNIDO, 2002). Hence, it is considered a challenge to maintain coordination with the duplication of regulatory activities of different government departments and institutions (FAO/WHO, 2005b).

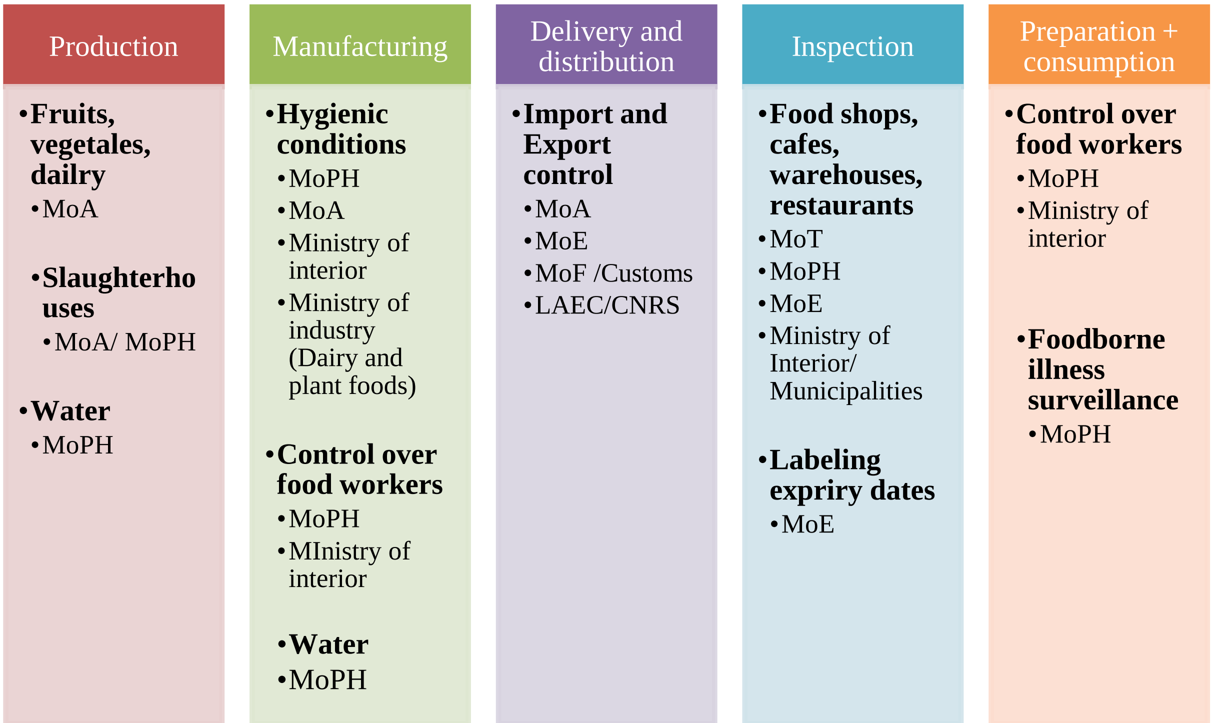


Figure 2.3. The functions of various national institutions along the food chain in Lebanon. Adapted from El-Jardali et al. (2014)

The main reason for the current overlapping responsibilities between each of the Ministries: the economy, agriculture, health, tourism, industry, internal (municipalities), the environment, and Finance (Customs) is not officially reported, nonetheless with the increased spate in reported cases of food safety violations, reported news and information by the General Federation of Trade and Labour Unions indicated that these overlapping responsibilities have developed over time as a result of a struggle for influence and power by different interest groups in the respective sector (Saleh, 2012). Ministries are not ready to take part for the benefit of the law that denotes the establishment of an independent food safety body (Box 2.1).

Box 2.1. Reported news and press conference

Saleh (2012): The problem is that each ministry wants to hold the bulk of the powers to its advantage. Food businesses are subject to multiple uncoordinated inspections as declared by Minister of Tourism.

MoE, (2014): The director general of the consumer protection department in MoE declared at that time that activation of a national control strategy for consumers 'protection shall be merely possible with adequate number of inspectors and the approval of the food safety law.

Husseini (2016): In a press conference (June 2016), the Minister of Public Heath disclosed the interception of ministry's decisions related to the food safety campaign by the governorate of Beirut and the north. In effect, there was objections of the ministerial decisions and intervention of judges in the course of the food safety campaign. In the opinion of governors, there is a lack for the role of public departments and municipalities in the jurisdiction according to law. Governor of Beirut considered that the public health department in the municipality of Beirut has the role to take the preventive measures in coordination with the MoPH. According to its authoritative role by law, only the municipality inspectors are entitled to sample foods and issue warnings.

Presently, the main regulations linked to food safety such as the food good manufacturing practices (GMP), additives, nutritional supplements, labelling are under the authority of LIBNOR, the Lebanese Standards Institution attached to the Ministry of Industry, which has solely the right to prepare, publish and amend national standards, as well as to grant the Lebanese Conformity Mark NL (FAO, 2016a). The standards include setting the dimensions, conventions, symbols, and the definition of products quality, as well as the methods of testing and analysis. They also include the codes of practice for professional and structural work. Most of the food commodities are required to follow the Lebanese standards, in cases where they are not issued, international Codex Alimentarius is the reference.

2.6. Implications of food safety problems on the ratification of the food safety law

A special parliamentary committee to deal with food safety that included several ministries was formed as a result of the food safety campaign led by the Minister of Public Health (WHO, 2015). In 2002, the committee submitted a food safety law proposal to the Council of Ministers to reform the existing laws and to unify the fragmented food safety system. The proposal recommended the establishment of a single government institution to govern all food safety stakeholders and adopt risk analysis for assessment, management and communication. It was stalled in the parliament until 2006, when the draft was debated in the Council of Ministers and referred to the Parliament's General Assembly for further discussion. But there has been no further action (El-Jardali et al., 2014). The importance of the revised law lies in the creation of the Lebanese Food Safety Agency (LFSA), which will be the referral body to implement and oversee the regulations, and implementations (El-Jardali et al., 2014). The LFSA is intended to be formed by a seven-member board of food safety experts from a variety of backgrounds and will oversee the implementation of a food safety law from farm-to-fork (i.e. farming, importing, exporting, packaging, storing and selling as reported in the news (Sidahmed & Semaan, 2015). Presently, there is no official information available on the law provisions.

Recent food safety scandals across Lebanon unveiled a series of food frauds that included entry of expired food products into the market. On 13 March 2012, 181 tons of spoilt meat were detected by an intensive inspection campaign by the MoE, in addition to 22 tons of expired meat imported from New Zealand, Australia and Brazil and tons of other imported expired goods intended for local market use (Diab, 2012). In 2014, the food safety campaign headed by MoPH continued to unveil serious food safety violations (El-Jardali et al., 2014; WHO, 2015) and brought about streams of negative news about food safety in Lebanon. Thus, the issue of food safety law was brought back into the cabinet agenda and ratified in 2015 (WHO, 2015). However, implementation decrees and enforceability have not been established yet.

2.7. Agricultural policy in the context of food safety

2.7.1. Sanitary and phytosanitary measures

Although Lebanon was one of the founding members of the General Agreement on Tariffs and Trade (GATT) (Irwin et al., 2009), it is still in the accession phase of the World Trade Organization (WTO) and in the process of drafting its legislations (Abou Ghaida et al., 2014). In 2009, the government undertook actions toward modernizing the laws and enhancing the legal framework as member countries in the WTO and Codex Alimentarius should comply with the sanitary and phytosanitary measures (SPS) and technical barriers to trade (TBT) agreements through integrating Codex standards in the national legislation (Al-Kandari & Jukes, 2009; WTO, 2016). The customs law in accordance with WTO was enacted in June 2001, which among others, simplifies procedures and introduces modern information technology for customs declarations and international standards for clearance.

It has established the SPS and the TBT enquiry points at the Ministry of Agriculture and the Lebanese Standards Institution (LIBNOR) in accordance with standards and guidelines of FAO/WHO Codex Alimentarius Commission for Food Safety, the World Organization for Animal Health, the International Plant Protection Convention for Plant Health (Abou Ghaida et al., 2014).

Several areas affecting Lebanon's ability to bring its legislation into WTO compliance were identified, and these included, the lack of conformity with WTO requirements on sanitary and phytosanitary measures, technical barriers to trade, import licensing and intellectual property (MoE, 2011).

2.7.2. Regulatory and policy framework

Initially, the food policy objectives of the Lebanese government aim to foster the role and impact of agriculture to the economy and were focused on assuring a steady supply of reasonably priced produce for the consumers by providing assistance and support to the local producers for facilitation of production and market linkages. It would also promote and enable agricultural conditions consistent with the agreements entered into with the EU and The Grain and Feed Trade Association (Markou & Stavri, 2005).

Policy instruments in Lebanese agriculture are mainly confined to direct payments, price guarantees and subsidies. Subsidy programs are common tools used by the government to boost farmers' productions and provide financial and technical assistance for market access, particularly international markets.

From 2000 to 2009, the agricultural sector received approximately 42 % of the SME development loans authorized by the Government (Markou & Stavri, 2005). However, the organization of marketing activities by farmers' associations and boards and food safety issues were overlooked. Besides the preferential considerations that were given to agricultural products, such as tobacco, wheat (Markou & Stavri, 2005), and recently to olive oil according to MoA decision 657 issued in 20 July 2011 to leverage the value chain (IDAL, n.d.).

In this context, the government has expanded a subsidy program on interest rates aiming at reducing the cost of borrowing for SMEs in the agriculture and tourism sectors. This approach resulted in an increased exports of Lebanese agricultural goods during the period 2010-2013, with an increase of 4 % between 2011 and 2012, and reaching 18% between 2012 and 2013 (MoA. 2014).

The loan-guarantee scheme, *Kafalat*, is another example of a policy instrument for Lebanese farmers that is intended to increase the capacity for lending to agricultural SMEs and stimulate investment.

There is neither similar financial support, subsidized loans nor any state guarantees available for the producers of fresh fruits and vegetables production. Further cooperatives are nearly absent and inactive in the production and marketing (Markou & Stavri, 2005) in view of a centralized system where regional and rural development initiatives are often managed by the central government (Michaels et al., 2010) hindering any potentially effective contributions of local government in the implementation and management of development programmes.

Between 2010-2013, the FAO project titled "Strengthening production and marketing of Lebanese agricultural products" was intended to develop institutional and organizational skills of the MoA for improving rural production, marketing and empower food security, quality system and safety practices (FAO, 2012a, 2016b). The project has contributed to improving institutional and operational capacities of the MoA and upgraded food safety and quality systems. Nevertheless, local inspection and control systems and review related legislations were still needed to improve food safety (FAO, 2012a, 2013). In this project, the GAP was addressed targeting three main crops: grapes, citrus and apples (Mhanna, 2016).

According to the Head of Rural Projects and Irrigation Department of the MoA, the execution of this project required the formation of a National Technical Working Group (NTWG) on GAP. The proposal for NTWG was drafted in 2013, but it is still not yet ratified. The role of the NTWG is intended to facilitate the adoption of international and/or national standards and regulations and guarantee systems for quality, food safety and environmental management by fruit and vegetable producers and other supply chain actors in Lebanon (Mhanna, 2016).

Furthermore, in 2009, the MoA framed an agricultural sector development strategy for 2010-2014 that articulated key areas in need for development plans. It included the mobilization of adequate financial resources, development of an appropriate legislative framework, and the strengthening of the MoA capacities and extension capabilities (MoA, 2014).

Subsequently, achievements reported for that period included updates made to regulatory/policy framework that addressed the issuing of a number of legislative texts (laws, decrees, decisions and regulations) to regulate handling of the different production inputs (fertilizers, seeds, agricultural pesticides, veterinary drugs) in addition to initiating control activities of domestic and imported food products according to international food safety standards and focusing on better quality, production, marketing and export of agricultural products (MoA, 2014).

Before the formulation of the new policy (2015-2019), an internal evaluation of agricultural and rural policy (2010-2014) was conducted by experts through the European Commission’s initiative for agriculture and rural development in the countries of the Southern Mediterranean (ENPARD) (CIHEAM/IAMM, 2014). The ENPARD report showed that progress was made in areas related to quality improvement of primary products which entailed reinforcing the current legal framework with more regulations and directives, e.g., pesticides levels on imported and exported products, the sanitary condition of transportation vehicles, capacity building for technical staff on inspection, HACCP, and improving the infrastructure). Despite, the progress was not sufficient to improve the quality and safety of Lebanon's products. It is reported that none of the Directorates in the MoA actually concentrates on consumer protection/food safety issues and on the inept extension and advisory services to extend technical knowledge on food quality and safety issues (Michaels et al., 2010). The quality control activities and conformity with the Lebanese norms were reported to be limited (Zurayk & Abou Ghaida, 2009) and the compliance to food safety requirements is not addressed sufficiently and critically in any reports.

SPS inspection systems were developed in terms of human resources, equipment and training. Nonetheless, shortage of financial resources and technical capacities did not allow effective implementation of strategy objectives (FAO, 2012a). The available information indicate that major efforts are still needed to implement a science-based effective food safety control system in addition to a great necessity for the promotion of irrigation techniques that increase water use efficiency and training farmers on better farming/good agricultural practices.

Thus far, the MoA formulated an updated Strategic Plan 2015-2019 based on the evaluation of strategy objectives and implementation for the succeeding years. This plan falls under the component "developing MoA capacities to better implement agriculture strategic orientations" which is one of the objectives of the Agriculture and Rural Development Programme, funded by the EU under the framework of the European Neighbourhood Policy. The policy strategy of the MoA and its evaluation indicated four key elements for future development of Lebanon's agricultural sector: crucial intervention in the reduction of its structural problems, improving the competitiveness of its agricultural products, better management of its natural resources and the promotion of a more coherent management of the territory.

The objectives of the strategy (2015-2019), among others are modernization of agriculture and increasing its productivity, efficiency and specialization, and ensuring competitiveness of major value chains in light of land fragmentation, small holdings, weak agricultural and marketing infrastructure. The strategy also aimed for the upgrading of sanitary and phytosanitary standards in conformity with international standards thus facilitating access to foreign markets in view of trade liberalization.

2.8. Equity to resources and extension services: Where does the local market stand?

2.8.1. Initiatives and schemes for agri-production to exports markets

Since 2005, various initiatives from the government and from international organizations were launched to enable local farmers to meet the food safety and quality requirements and improve their access to international markets. Overhauled by a relatively interrelated web of international organizations, farmers could overcome barriers of stricter requirements imposed by international markets.

It is evident that products destined for the EU should meet the standards requirements of the importing countries and hence, safely produced by their measures. In the context of export support, the Lebanese government appointed international companies to ensure the quality of agricultural products meets the standards of the EU, the Gulf countries and the United States (ESCWA). Similar substantive efforts of economical extension and initiatives are not known for the safety of fresh produce for domestic consumption.

During that period, the government has embarked on the realization of several objectives enhancing export activities and reinstated the Export Plus Programme in 2011, a government subsidy program implemented by the Investment Development Agency for Lebanon (IDAL), with an annual budget of LBP 50 billion (equivalent to USD 33 million). It aims to increase agricultural exports and improve the quality of agricultural products by assisting exporters with their crops (vegetables, fruit, flowers and eggs).

While Lebanon was known to have relatively low levels of spending on food quality and safety programs, this is different with IDAL export programmes and takes place tender processes (WTO, 2008). IDAL applies incentives to maintain safety and quality of products by providing partial reimbursements of the transportation costs of Lebanese exporters of fruit and vegetables provided they comply to some quality standards (IFAD, 2008).

Several USAID funded projects, such as Sustainable Agro-Industry in Lebanon (ASAIL) and Agricultural Quality Control and Certification (QCC) programs, were implemented via an international organization, such as ACDI/VOCA, an economic development organization, as part of the strategy to strengthen the competitiveness of the agricultural sector. Between 2005-2008, ACDI/VOCA's Action for Sustainable Agro-Industry in Lebanon (ASAIL), a 2½-year, $6.9 million program funded by USAID, was launched to address the development of two main subsectors, the niche Lebanese foodstuffs and small ruminant (goat and sheep) dairy products (ACDI/VOCA, 2013). The importance of these programmes lies in their focus on enabling farmers' linkages with markets, assistance in meeting sanitary requirements, administrative formalities, post-harvest requirements and marketing conditions; ensuring export of agricultural products is facilitated at the borders. More so, Action for the Modernization of Agriculture and Rural Areas program adopted an integrated value chain approach to expand the market or forage crops and fruit crops considering their substantial contribution to market potential.

ASAIL implementation was also based on a value chain approach to develop Lebanese foodstuffs and small ruminant dairy products sectors which boosted the incomes and profitability of SMEs and cooperatives. QCC touched on an important pillar of the quality control by building the capacity of food-testing laboratories and product-development plants established alongside ASAIL program to provide services essential to SME agro-processors and producers. At the same time, it serves as an information gate, called the agro-products site directory for international export requirements, or TASDIER (in English: export), for famers inquiring on regulations and standards to access export markets. The database is currently managed by the Federation of Chambers of Commerce, Industry and Agriculture in Beirut.

Lebanon Business Linkages Initiative is another project, a $4,725,405 project funded by USAID under the FIELD Leader with Associates program which facilitates communication between U.S. importers and Lebanese exporters/processors and provides guidance to market driver firms on packaging and labelling, U.S. specialty food market requirements, sales sheets and brokering. The resulting achievements equalled to a $4.85 million increase in the value of international exports of targeted sector commodities (ACDI/VOCA, 2013).

Focus on horticulture and small livestock continue through Farmer-to-Farmer (F2F) initiative which was initiated for the period 2008-2013 to enhance the country's horticulture and livestock industries and assisting producers in improving the fresh fruit and vegetable productions and integration into the horticulture export value chain through enhancement of farms practices and post-harvest handling.

2.8.2. Constraints reflected on the safety of the domestic fresh produce market

There are various problems that hinder the development of the agriculture sector and supposedly affect the safety of fresh produce. Lack of policies and underdeveloped regulations to meet up with the emerging risks can have direct influence on the safety of fresh produce, such as the lack of policies or incentives for adequate management of agro-industrial waste of olive residues from olive mills, effluents from poultry farms, and slaughterhouse (IFAD, 2008) that certainly carry a high risk of pathogen contamination on fresh produce. Consequently, the local production and distribution market of fresh vegetables suffer from the lack of law enforcement and the domination of large retailers (CTCS, 2010). At present, Lebanon does not have regulations and a specific policy on the implementation of GAPs, and operational decrees for existing laws is lacking (Farajalla et al., 2015). In 2010, the concept of GAP was addressed in several policy papers. Some measures have been implemented and projects were developed locally especially focused on the introduction of the Integrated Pest Management and Integrated Water Management in the MoA policy (Mhanna, 2016).

As larger scale commercial operations serve export markets and benefit from export programmes, the domestic distribution market is served predominantly by small and medium scale farmers and affected from the low compliance with the food safety standards and international requirements, a lack of marketing regulations, and competition from lower-priced products from neighbouring countries (FAO, 2012a). Added to this, there is a lack of adequate facilities for storage, grading and packing of agricultural products as one of various constraints (Chalak & Sabra, 2007; DAI, 2014). In this context, Lebanon's accounts revealed relatively low levels of spending on food quality and safety programs. Nearly US$ 1.1 million was spent in 2007 on these initiatives through IDAL's export plus program. This is equivalent to 4% of MoA budget for the year 2007 (WTO, 2008).

However, not captured by the existing reports of the Lebanese market, large producers are overtaking the local domestic distribution chain whether through leasing or owning growing lands. Lack of governmental control as well as political and economic interests have favourably fostered the emergence of monopolizing role of middlemen and traders of agricultural inputs, marginalizing small farmers' businesses. The latter being affected by a fragmented market and poor marketing infrastructure (Chalak & Sabra, 2007). Small farmers have limited means to access funds; 73% of Lebanese farmers have a plot of less than one hectare which reduces any chances of their credit worthiness and access to other agricultural inputs or exportation programmes (IFAD, 2011). At the same time, the cooperatives are currently not actively engaged in production or marketing the relevant produce (Markou & Stavri, 2005).

Following interviews with small farmers in Bekaa, a local newspaper Al Hayat daily reported about the problematic monopoly of middlemen who control the domestic marketing and export of agricultural produce and hence collecting the benefits due to their power and influence (Al-Hayat, 2015). Farmers indicated that traders buy directly from the field at very cheap prices to sell at much higher prices at wholesale markets and even higher to distributors. The dealers often attribute the low price to the poor quality. It was noted that such shortcomings were a result of inadequate agricultural extension services and the absence of support from public institutions. Until this date, effective extension programs are still considered limited (Census 2010, 2012). According to the Head of Department of Rural Project and Irrigation in the MoA, there are 28 extension services centres in different regions responsible to extend support to farmers that lack sufficient human resources. Normally, one or two agriculture engineers exist per centre. The agriculture sector is not attractive in view of the low income, which also reflect on the commitment levels of employed engineers. Since 2005, Lebanon has no updated or approved national budget and the last national budget did not cover these areas. Hence, the sector relies on funds that would not provide a sustainable solution (Mhanna, 2016). This is confirmed by Farajalla et al (2015) who reported that the Lebanese government is still functioning based on the national budget developed in 2005 which was set according to different needs, hence did not consider the recent economic inflation.

Currently, there are no regulations or national standards for good agricultural practice to be enforced or implemented by farmers. Promotion of good practices in relation to integrated pest management are realized through the extension services (Mhanna, 2016).

The influence of political marginalisation of the agriculture sector in Lebanon on the monopolizing control of traders over the marketing structure, imports of fertilizers, pesticides, and other agriculture inputs was reported. Thus, middlemen are reaping most of the benefits while small farmers receive a very small portion of no more than 20%. Those middlemen are actually well connected by interests with large producers (Al-Akhbar, 2014).

2.9. The central wholesale market in Beirut

There are two main wholesale markets in Beirut (Sin el Fil and Sports City). The biggest wholesale market in Lebanon is the Sports City market in South Beirut, with 80 stalls supplying the vast majority of grocery shops and restaurants. Less than half of the stalls have cold storage with limited capacity confined to keep the inventory from spoiling for several days (DAI, 2015).

The wholesale markets are basically a collection of stalls for different categories of producers, packers/exporters who buy from middlemen, small and large farmers, and trade volumes at the wholesale level. They are run by associations of traders who occupy the designated spaces, collect rent for the warehouses, and organize cleaning and security. Large wholesalers in the market are owned by independent exporters/importers who also operate own washing, packing and cold storage facilities outside the urban areas, as shown in the field work during the present study and reported by DAI (2015).

2.10. The interrelation of the political will and food safety

Many of the regional and rural development initiatives are highly centralized and managed by central government, with limited contribution of the local government and community due to opposition from the political establishment, scanty financial resources and limited human resource capacity (World Bank, 2004).While access to subsidies and loans proved to be an incentive for development and expansion, structural problems persisted as barriers, i.e. low prices for producers, huge profits for the marketers/middle men (Markou & Stavri, 2005).

Despite the steps taken by the MoA whether locally or regionally funded, the development of the sector is subjected to impediments stemming from a weak public sector (structural, organizational, regulatory, poor infrastructure). There is a lack of public funding, weak expenditure mechanisms (with less than 1% of the state budget) as well as limited bank loans. The budget allocated to the MoA never exceeded 0.5% of the national budget per year (Chalak & Sabra, 2007) and public spending in agriculture is highly fragmented (Michaels et al., 2010). As such, farmers suffer high costs of inputs, labour, and energy, degradation of natural resources; and low competitiveness of the agricultural products (MoA, 2014), in addition to various social challenges related to lack of farmer status under the labour act, which does not contain any specific provision for farmers, high unemployment rate among youth and continuous decline in workforce in rural areas. The growing urbanization resulted in the reduction of lands and increased cost of agricultural areas, small and fragmented lands, holdings and inadequate zoning of rural and urban regions. In parallel, long-standing constraints to development in the agri sector (i.e. low productivity, inequality in ownership and access to productive assets, rural poverty, lack of access to irrigation networks, agricultural roads, weak marketing infrastructures and poor logistics and infrastructure development) are obviously not resolved (FAO, 2012a). The deficient policies and poor coordination among stakeholders led to a widespread use of foreign labour, insufficient knowledge of modern techniques and environment-friendly practices, excessive use of pesticides and inappropriate use of fertilizers which hinder any rapid progress in accordance with international standards (Markou & Stavri, 2005; Sheehan & Abdul Latif, 2008). Failure in implementation of laws and enforcement were attributed to the absence of one enforcement body, accountability mechanisms and the presence of corruption (Farajalla et al.,2015). Together with the poor standards and the threatening environmental practices related to pollution from haphazard dumping of slaughter waste and waste from animal farms, inadequate as well as inequitable investments in irrigation infrastructure (Markou & Stavri, 2005; Farajalla et al., 2015) pose further constraints for an effective safe production and hypothetically a great health risks related to microbiological hazards on fresh produce. Despite of the support from international organizations, efforts to surmount those constraints are not sufficient to prevent emerging diseases that might be linked to fresh produce in Lebanon and which are documented increasingly as global health concern (Buchholz et al., 2011). Investigations of several human infections related to fresh produce consumption pointed to the early stage of production as the major source, e.g., contaminated irrigation water, unsanitary conditions in the farm’s processing shed, application of manure (CSPI, 2015).

Pollution of most water resources is asserted by experts and scientist (Jurdi, 1992; Darwish, 2004; Houri & El Jeblawi, 2007) and widely publicized in the local media (Hamieh, 2011; Shaheen, 2014). According to a 1998 study by the National Council of Scientific Research of Lebanon for UNICEF 60 to 70% percent of all natural sources were affected by bacterial contamination (Jurdi, 1998). The Litani and Al Assi (Orontes) rivers that irrigate the cultivated areas of the Bekaa valley, are reportedly contaminated with bacterial and chemical effluents from industrial and household leaches and raw sewage. Natural resources as major inputs in agriculture and its management is becoming more complex as to its mobilization, storage and distribution, inefficient management, unregulated permits and sinking of private wells and over-exploitation (CIHEAM/IAMM, 2014; Farajalla et al., 2015). Farmers with limited financial resources and in rural areas struggle with adopting modern irrigation techniques. The problem of water availability is at its highest peak during the dry season, from July to October, and might worsen in the future as a result of climate change (IFAD, 2011).

The former Minister of Agriculture justified the existing state of the agricultural sector as an outcome of governmental economic policies for the past twenty to thirty years (Daily star, 2014).

The development of a competitive and safe agriculture environment in Lebanon is predominantly hindered by political conflicts and lack of any internal consensus among different parties (Chalak & Sabra, 2007), which impede any chances for institutional reform as indicated by Lebanese Transparency Association (2011). The latter classified Lebanon as the 134th most corrupt country in the world, out of the 183 countries. The World Bank's Worldwide Governance Indicators (WGI) also ranks Lebanon in the lowest quarter of the percentile on a scale from 0 to 100, in terms of control of corruption (Figure 2.4), which according to a report of the World Economic Forum (2012), affect decisions in investments and trust in government ability to manage the country affairs fairly. The recent report by Farajalla et al. (2015) shed light on the influence of corruption in monitoring agencies on the water resources management that is also related to water in agriculture sector in Lebanon.

The corruption comes in the form of bribes for the customs, the registry and permit service, the police and the judiciary being the most common bribe takers. It is thought to be a result of lack of regulation of political party financing and accountability (Global Integrity, 2009; Farajalla et al., 2015).

The judiciary institution which role is to foster the law is not independent of the direct political interference in Lebanon (Freedom House, 2012) that actions taken against food frauds or safety violations are never long-term and in many cases lacking (El-Jardali et al., 2014; Farajalla et al., 2015; Organization of Consumer Protection, n.d.). Consequently, Lebanon failed to establish accountability and equity systems (Figure 2.5) which are also reported as major constraints in the water sector (Farajalla et al., 2015) and encourage substantial violations and protect the accountable by political layers. The 2014 food safety campaign launched in response to serious food safety violations ended up with closures of incriminated institutions. However, juridical decisions were not effectively implemented, which was reconfirmed in a press conference by the Minister of Public Health (Box 2.1).

The disclosure of food safety violations that are threatening consumers' health should have been the turning point for the legislative body to endorse the food safety law.

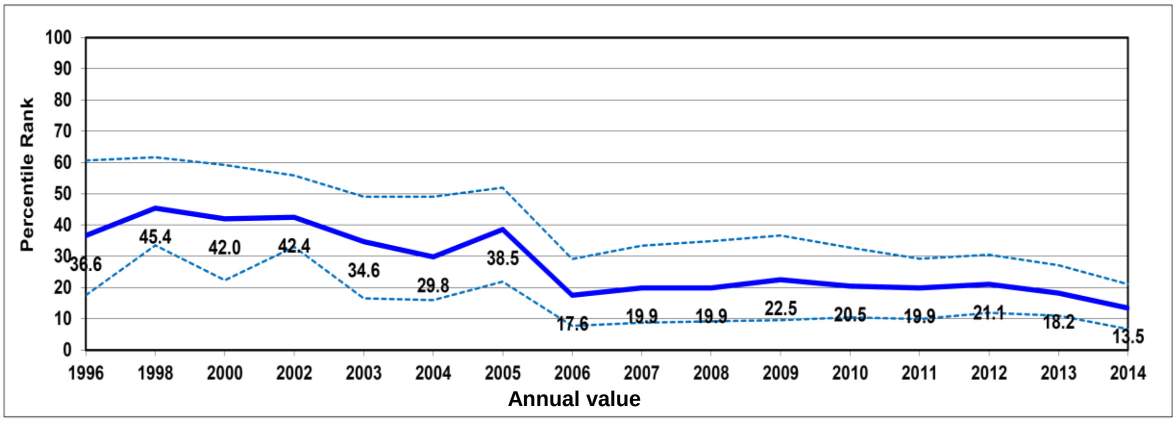


Figure 2.4. Aggregate Indicator: control of corruption –Lebanon (1996-2014)
Source: World Bank

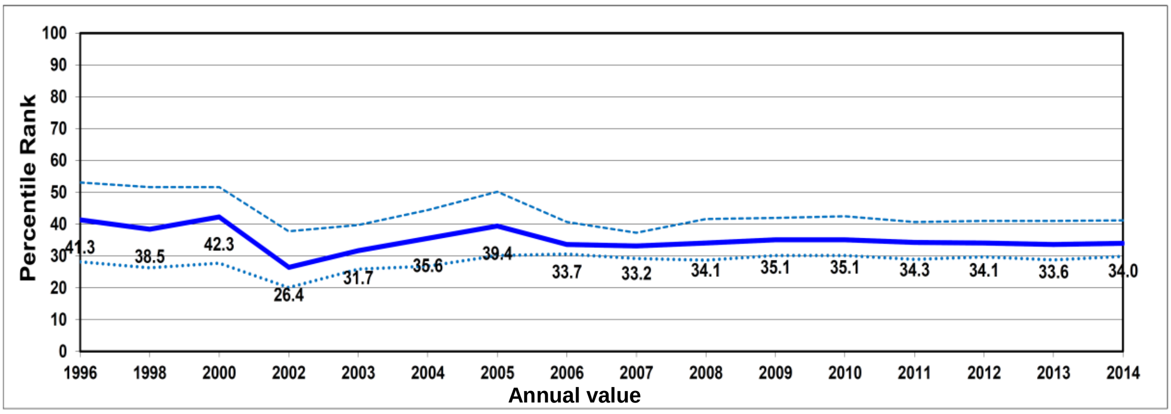


Figure 2.5. Aggregate Indicator: voice and accountability –Lebanon (1996-2014)
Source: World Bank

In conclusion, the existing political structure and institutional role, and to a lesser extent the preoccupation with regional uncertainties, have major impact on the course of food safety governance in Lebanon, particularly in relation to locally produced, marketed and sold fresh produce. The existing structure is explicitly described by Batniji et al. (2014) as she stated "What distinguishes the Arab world - almost as much as the Arabic language itself - is the absence of political accountability throughout the region. Across the Arab world, political systems are often dominated by elites and do not have ways to address the population's broader concerns, showing the neglect of operative methods for accountability".

Food safety governance in Lebanon is currently hampered by fragmented regulatory texts and enforceability that are far from sufficient to address the needs and safety of the entire food chain as a result of a political deadlock (Farajalla et al., 2015). Food safety protection for consumers require strong government regulations and will to address underlying issues (FAO/WHO, 2003).

The present analytical work showed that the Lebanese plural system of double standards in governing food safety conflicts with the main goal of an adequate national food safety system that is suitable for the protection of all consumers equally from food hazards.

It presented information and evidence on the absence of a clear national policy to protect local consumers' health amid the stringent requirements and the extension services and support available to farmers for improving accessibility to export markets. This in turn permits violations and uncontrolled unsafe practices as encouraged by lack of accountability and economic needs, profits and drive to access resources (Farjallah et al., 2015).

To further support this argument, an empirical investigation of the food safety practices and compliance levels in the agriculture environment of key producers and along the supply chain is described in the next chapter. The latter will provide additional insights into the implications of the existing conditions on locally marketed fresh produce.

3. Understanding the routes of contamination of ready-to-eat vegetables in the middle east: Lebanon an exemplary model

3.1. Introduction

In the Middle East, many types of vegetables are eaten raw in salads or used as garnishes in appetizers and traditional dishes, and increasingly because of their perceived healthy attributes. Yet, they have been in recent years a major contributor to foodborne illnesses in other parts of the world (Lynch et al., 2009; Painter et al., 2013; Callejón et al., 2015). In the US, leafy greens were identified at the top of the 10 riskiest foods regulated by the Food and Drug Administration (FDA) accounting for almost 40% of foodborne outbreaks based on data derived from the CDC (CSPI, 2009). Pathogens identified as hazards on fresh vegetables include S*higella* spp., *L. monocytogenes*, *Staphylococcus aureus (S. aureus), Aeromonas hydrophila* and the spore-formers *Bacillus cereus*, *Clostridium botulinum* and *Clostridium perfringens.* However, the ones implicated in most outbreaks involving fresh fruits and vegetables are *Salmonella, E. coli* O157:H7 (Buck et al., 2003; European Commission, 2002) with reported doses as low as 10 cells and 2–2,000 cells, respectively (Harris et al., 2003; Kisluk et al., 2012). Norovirus is also among the pathogens of greatest concern that are associated with fresh produce outbreaks (Todd & Greig, 2015) and the high likelihood of inflicting illnesses is attributed to its low infectious doses10-100 viral particles as reported by D'Souza and Su (2010) and Barrabeig et al. (2010). The reportedly held rationale that increased consumption of fresh vegetables is actually the reason for the increased foodborne illnesses has been challenged in the American Society for Microbiology (2008) report stating that the proportion of outbreaks due to leafy greens has increased beyond what can be explained by increased consumption. This emphasizes the focus on the primary production stages on farms and subsequent processing as the main contamination sources, although no doubt coupled with enhanced epidemiological and surveillance programs (CSPI, 2009) and the expanded interaction of the local and international markets of fresh produce.

Perishable fruits and vegetables are now transported long distances from growing to retail markets with a wide product distribution range to meet consumer demand. Thus, any associated illnesses could be widely dispersed within or beyond national borders, requiring sophisticated surveillance tools like PulseNet to identify these, while traceability to origin remains a challenge in such an extended supply chain (Sivapalasingam et al., 2004). This may be beyond the resources of many developing countries including those in MENA, where illnesses related to leafy greens may be underestimated or rarely reported. In this region, prompt concerted research efforts to understand, prevent and control risks of illnesses arising from consumption of contaminated salad vegetables and fruits are lagging behind those in other regions. Throughout the farm-to-fork continuum, fresh produce is subjected to numerous opportunities for microbial contamination due to a range of handling, processing, storage and transportation activities which in the event of unfavorable conditions may lead to the presence of microbial hazards (Gil et al., 2015).

Water is recognized as one of the most important vectors of enteric human pathogens on vegetable crops (Park et al., 2012), This is exacerbated by the fact that water scarcity impacts the quality of the water used for irrigation coming from uncertain sources which may harbor pathogens (Leifert et al., 2008). Facing multiple challenges, i.e., political, economic, climate change, unfortunately many developing countries are increasingly reverting to the use of untreated wastewater for irrigation and processing of vegetables (Aiat Melloul & Hassani, 1999; Thurston-Enriquez et al., 2002; Ensink et al., 2007; De Bon et al., 2010; Castro-Rosas et al., 2012; AL-Jaboobi et al., 2013). One example for this is the produce industry in Lebanon, where agricultural production is concentrated in the Bekaa Valley, both the most cultivated area and the most affected by water pollution (Jurdi, 1992; Halablab et al., 2011).

Almost 146 farms use the surface water of the Litani River to irrigate various vegetables as reported in 2011 in local news (Hamieh, 2011). This river is frequently polluted by untreated sewage, domestic solid waste, and industrial effluents (Houri & El Jeblawi, 2007) and as result, leafy greens in that area have been reported to pose health risks to consumers (Halablab et al., 2011). In addition, export potential for produce may be increasingly at risk because importing countries are demanding higher standards. Despite the fact that risks of foodborne illness are likely to be higher in the developing countries of the MENA regions where the waste water treatment is still underdeveloped and use of untreated water for irrigation is illegal, most research on the microbiological safety of fresh vegetables and fruits has been carried out in developed nations (Johnston et al., 2005; Lehto et al., 2011; Seow et al., 2012; Allen et al., 2013; Wood et al., 2015). Certainly, very little has been done in Lebanon (Halablab et al., 2011; Khatib et al., 2015), maybe because the surveillance data for foodborne illness is lacking, and partly because of lack priority for research funding. There can be no doubt that foodborne infections originating from contaminated fruits and leafy green vegetables do occur in the MENA region including Lebanon, based on surveillance data from other regions since they are frequently eaten at most meals (European Commission, 2002; Painter et al., 2013; EFSA, 2014).

To address this lack of understanding of what and how microorganisms of concern are transmitted across the food chain, this study was conducted to determine the risk factors contributing to microbial contamination of vegetables eaten raw, represented by flat leaf parsley (*Petroselinum crispum*. var. *neapolitanum*), romaine lettuce (*Lactuca sativa* L. var. *longifolia*), and small red radish (*Raphanus sativus*) from farms in the Bekaa Valley, Lebanon, to the central market of fresh vegetables in Beirut.

3.2. Materials and methods

3.2.1. Study design and sample collection

3.2.1.1. On-farm and supply chain assessment instruments

A structured questionnaire was designed based on GAP audit checklist from the University of Idaho Potato GAP program, with slight modifications (Kimberly Research and Extension Center, n.d.). The checklist was designed for fruits and vegetables in general and in fulfilment of requirements to inspect growers for provisions of USDA GAP and GHP audit procedures. The questionnaire comprised a set of questions that covered demographic information and farm profile, in addition to a number of areas related to agricultural practices as presented in APPENDIX C. In general, the different sections of the questionnaire comprised closed questions, multiple-choice questions and statements rated on a five points rating scale.

The interview was combined with photographs taken upon participants' approvals. There were cases when photos were not permitted.

The onsite-assessment for GAP was planned to be conducted on the same sites where produce samples were collected for microbiological analysis.

3.2.1.2. Sampling procedure and recruitment of participants

In general, a purposive sampling technique was employed in view of its suitability in answering specific research questions and testing specific hypotheses. A purposive or judgmental sample is a non-random sample in which the units of observation are selected based on a pre-established criterion and "researcher's judgment about which ones will be most useful or representative" (Babbie, 2010). It was intended to focus on major players in the supply chain recognized in the market by their large production capacities. Thus, the information generated from their farms and the distribution chain shall be of significance.

In view of the limited information on the internet and lacking formal addresses of farms, a snowball sampling was performed by being informed by one producer to the next and so on (Vogt, 1999). Informal interviews with operators of stalls in the central wholesale market were conducted (one of the two main markets in Beirut) to develop a list of producers' names and locations. The wholesale market of fresh produce is located in south Beirut and one of two main wholesale markets of fresh produce in Lebanon. It is the main source of produce to a great majority of restaurants, groceries, retails, and hospitality industry in Beirut (DAI, 2015). Ten major producers of fresh vegetables (leafy greens, tomatoes, radish, etc.) and two washing facilities located in Bekaa area and linked to private stalls in the wholesale market in Beirut were selected. The Bekaa valley was chosen for that agricultural production is predominantly concentrated there, and known to be heavily affected by water pollution as described in chapter 2.

The sample size was determined by,

i) The fact that the market is monopolized by middle traders and large producers who marginalized small farmers and generally dominate the wholesale market. Hence, the focus was on leading producers of fresh vegetables in assessing compliance to safe practices in attempt to explore underlying factors other than resources or poverty.

ii) The rapid escalation of insecure conditions at the borders due to international conflicts which restricted additional visits to the area during the period of the study.

The selected participants are producers renowned in the local market and at the same time exporters of fresh produce. They are in control of the whole chain of production; starting from owning or renting agricultural lands, owning the packing and washing facilities and transport vehicles down the chain to stalls in the wholesale market in Beirut. This information (producers' characteristics) was determined during preliminary interviews with stalls owner in the wholesale market and field visits conducted at every stage of the chain (Table 3.2). Such characteristics of the agriculture products market and key intermediaries were also documented in the USAID project on value chain (DAI, 2015)

The basic structure of the fresh produce chain consisted of growers, wholesalers and retailers (Figure 3.1). The growers own the lands and others are renowned large producers who own and lease farm lands. In some cases, producers own the washing and storage facilities for local distribution and exportation, and are among the wholesalers in the central market.

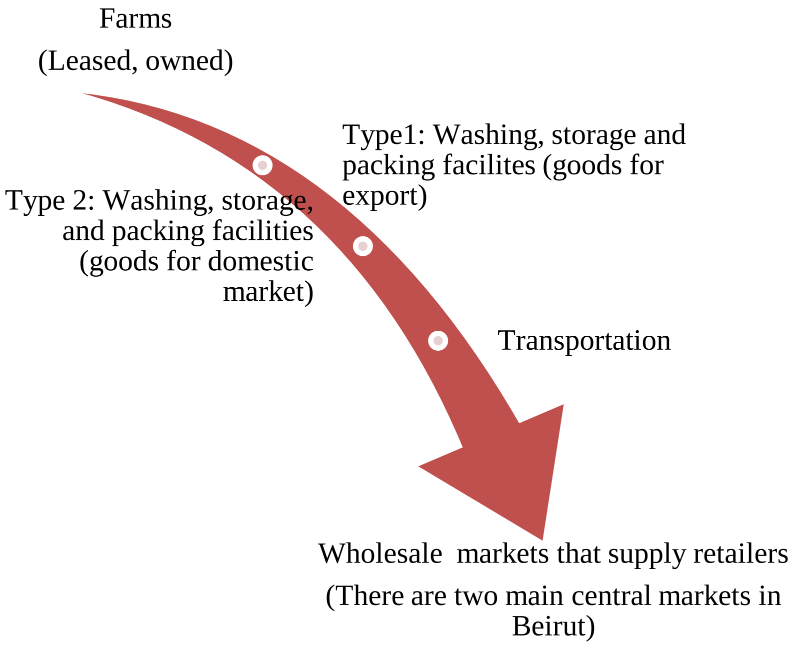


Figure 3.1. The basic structure of the fresh produce chain

Farmers were invited by phone to participate in this study, in other cases. For some, it was not easy to reach out by phone, a CITI certified research assistant (Agriculture graduate) established contact by visits on fields to introduce this research with the consent forms.

3.2.1.3. Fresh produce and data collection

Samples of fresh produce commonly eaten raw, i.e., lettuce, parsley and radish, (n=90) were collected in July-August 2013 and July 2014, a relatively hot and dry season in the Bekaa. Sampling areas included the surveyed farms (n=10), crop washing facilities (n=2), and the wholesale market in Beirut. It was planned to select items from washing facilities and wholesale market stalls that were traced to the source (surveyed farms) in order to assess the trend in the farm-to-retail contamination and microbial growth on fresh produce. Table 3.1 shows samples distribution across different sampling locations.

The sampling strategy on farms was based on the requirements of FDA's Pesticide Analytical Manual (FDA, 2015) and the FAO guidelines (FAO, 2009). Those manuals were developed to examine pesticide residues for regulatory purposes and adopted for field sampling for microbiological analysis. Fresh produce was sampled from different points of the plot in an S pattern, leaving 1 metre at the edges and ends of rows. A whole head of lettuce, and a bundle of parsley or radishes was considered as one sample. The number of sampling points was based on the sample size of the crop and its availability. In the packing/washing locations and wholesale market, three pieces of each type of crops were selected from top, middle and bottom of each basket randomly selected from various stalls owned by the participants (producers). In general, the sample size was determined based on the project timeframe and availability of funds.

Water samples (n= 5 of 1 litre-samples each collected in 250 ml portions from different points of the crop washing ponds or in 1 litre bulk from the wells and n=6 of 100 ml samples from water streams) were collected in polystyrene sterile bottles/cup.

Samples were placed in insulated coolers with ice-packs and transported 135 km to the laboratory the same day. Logistically it was not feasible to process the food samples on the same day, hence they were stored in freezers at -18ºC to be analysed on subsequent days, whereas the water samples were analysed that day.

Table 3.1. Summary of fresh produce and water samples collected from different points of the agro-food environment

Sample sources	Type of samples	Label*	n (%)
Farms fields	Fresh produce†	F-FP	35 (38.9)
Post-harvest washing ponds	Fresh produce	PHW-FP	15 (16.7)
Wholesale market	Fresh produce	WSM-FP	40 (44.4)
Total			**90 (100)**
Wells	Irrigation water	W-WI[1]	30 (53.6)
Post-harvest washing ponds	Crops washing water	PHW-W[1]	20 (35.7)
Water streams	Irrigation water	Water streams	6 (10.7)
Total			**56 (100)**

[1]Water samples analysed in 100 ml volumes
*The abbreviations listed under "Label" are used in subsequent tables and texts
†Type of fresh produce samples included lettuce, parsley and radish

3.2.2. Microbiological analysis of samples

3.2.2.1. Counts of microorganisms' indicators

For irrigation and wash water microbiological assessment, *E. coli* designated as Hygiene Criterion indicating faecal contamination (EFSA, 2014) and total coliforms (TC) were tested. The group TC comprises the genera *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*, indicator organisms that indicate the general sanitary level of water and possible contamination by different pathogens (WHO, 2006; Pachepsky et al., 2011). The enumeration of bacteria was performed according to the filtration method following EN ISO 9308-1:2000 using selective enrichment and RAPID'*E. coli* chromogenic media (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK).

Fresh produce samples were analyzed for the presence of pathogens and hygienic indicator organisms, i.e., *S. aureus*, *Salmonella* spp., *L. monocytogenes*, and for total plate counts (APC) and. *E. coli* and TC (WHO, 1989, 2006). APC were included as an indicator of any microbiological pollution and of existing favourable conditions for the multiplication of microorganisms. This parameter is useful to indicate efficient applications of GHP and temperature control during processing, transportation, and storage (Aycicek et al., 2006). Given a reported high counts of *S. aureus* on vegetables cultivated near the Litani River (Halablab et al., 2011), its frequent recovery from waste water and abundance in the animal production environment particularly in chicken litter in other countries (Schilling et al., 2012; Hashem et al., 2013), *S. aureus* was also considered in this study.

For microbiological analysis, all the media used were obtained from Bio-Rad Laboratories Ltd., Hemel Hempstead, UK unless otherwise stated and samples were analysed according to ISO 16140. Briefly, 10 g of the samples was weighed into sterile stomacher bags and homogenized with 90 ml sterile buffered peptone water (BPW) for 2 min at medium speed. Samples of 100 µl of each of the 10^{-1}, 10^{-3} and 10^{-5} dilutions were spread on in duplicates on appropriate media. APC were enumerated on plate-count agar at 37ºC for 48 hours. As for *E. coli* and TC, 1 ml from each decimal dilution was dispensed into petri dishes for enumeration by pouring technique using RAPID'*E. coli* 2 agar. The plates were incubated at 37ºC for 48 h. For the detection of *S. aureus*, typical presumptive colonies with clear halo resulting from proteolysis of egg yolk were further tested using a latex agglutination test (Pastorex Staph Plus). *Staphylococcus aureus* was enumerated on RAPID'*Staph* Agar supplemented with egg yolk. Typical colonies on the plates were enumerated and colony counts in 1 g sample were determined. The counts were reported as means of colony-forming units (CFU) per g and were converted into log CFU/g. *Salmonella* spp. and *L. monocytogenes* was reported as present or absent.

3.2.2.2. Detection of pathogens

For the isolation of *Salmonella* spp. and *L. monocytogenes*, the pre-enrichment/enrichment selective plating method was used according to ISO 16140. In the case of *Salmonella* spp., selective enrichment was performed in Rappaport-Vassiliadis-soya broth to be incubated at 41.5ºC. After 24 h of incubation, a 100 µl sample was plated on RAPID

Salmonella agar and plates were incubated at 37 C for 24h (± 2h). While for *L. mono*cytogenes, Fraser ½ broth was used in the selective enrichment and after incubation for 1 h at 20ºC, 100 µl of the homogenate was transferred onto RAPID'*L. monocytogenes* agar plates to be incubated at 37ºC for 24–48h. Typical *L. monocytogenes* colonies were afterwards selectively identified. *Salmonella* spp. colonies were identified biochemically by the lysine iron agar and tryptic sugar iron agar slants biotyping technique. Additional confirmation for positive *Salmonella* spp. colonies and for *E. coli* was done by the API 20E bacterial identification test strip, and for *L. monocytogenes* by the *Listeria* API strip (bioMérieux, Marcy l'Etoile, France).

3.3. Statistical analysis

Descriptive and frequency tests were performed using Windows version of SPSS 21. Bacterial counts across different points of the supply chain and in different types of produce were analysed. Kurtosis Levene's test for homogeneity variance showed normality within the distribution of the CFU counts, except for *E. coli* that showed non-normality which violates one of the assumptions underlying analysis of variance (ANOVA). In this case, Kruskal–Wallis tests was used for groups comparison, while when it is tenable, the mean values were compared by one-way analysis of variance (ANOVA) and subject to Tukey test to determine any statistically significant difference ($p < 0.05$) among the means (Granato et al., 2014). Chi-square Fisher exact test and non-parametric correlation (Spearman's rho test) were applied to test associations and correlations among bacterial counts and categorical variables. Linear regression analysis was performed to test the predicting power of agricultural water of the hygiene criteria in fresh produce.

E. coli prevalence was calculated by using the number of samples tested positive for *E. coli*, and then dividing that number by the total number of samples.

3.4. Results

3.4.1. On farm assessment and observation survey

The interviews and observations at the sampling locations identified a critical lack of good agricultural and post-harvest practices and several potential sources of contamination. Figure 3.2 illustrates the multiple stages along the fresh produce pathway observed during the survey. The 10 major farms reported that they do not implement GAP. Overall, producers reported not to have been trained neither on GAP that encompass composting, soil and fertilizers management nor on GHP and that included all labourers. It is common that land owners rely on the experience of seasonally hired poor uneducated labourers for farming and harvesting. Overall, there was a complete absence of workers' health and hygiene policy. This issue was not monitored or controlled by local authorities. Often, lodgings tents were located nearby crop fields, and the sewage treatment plants and adequate sanitary conditions were generally lacking. The unawareness of workers on the fundamental requirements of adequate sanitary conditions and hygienic practices was evident during the interview. Producers also indicated that agricultural water and soil were exposed to grazing animals i.e., sheep, further to the close proximity of farming fields to landfills and chicken farms (Table 3.2). There were cases when produce was growing close to land used for chicken and livestock production or landfill and manure storage, while mechanisms for treating chicken manure to reduce expected levels of pathogens was mostly based on the "experience" of farmers. All farms used sprinkle irrigation system and depended mainly on surface water coming from the Bardouni streams mixed with untreated sewage water from nearby villages, however others had private wells (Table 3.2). At the same time, traceability and coding was generally not applied, it was rather based on a primitive way and limited knowledge as to its meaning; one farmer reported identification of batches by volume and quality. Two of visited farmers (SF02 and IB) owned and operated two different post-harvest washing facilities that differed in the hygiene standards. The facility used for washing products aimed for exportation business seemed to be operated under better hygienic conditions (i.e. disinfection of fresh produce), the other was used for local production. As noted during the observations, hygienic operations in washing/storage stages and cold chain transportation were deficient. Cleaning of plastic crates and implements in the storage areas and washing facilities were reported as poor. At the same time, the producers reported an unsatisfactory clean environment and lack of disinfection applications throughout the washing processes. Unawareness of personal hygiene of workers engaged in washing the fresh produce was recorded. This was manifested by unclean personal clothing, lack of washing sinks and young children observed stepping into the ponds to assist in removing soils by dipping and agitating the radish or parsley manually inside the water.

Four major sources of irrigation water were identified i.e., wells, river, artificial ponds and sewage. It was noted that non-potable river water was used for irrigation and post-harvest washing. However, when water sources declined in the summer, farmers were forced to use private wells for irrigation and filling the washing ponds. In two of the farms, sewage water was used both as irrigation and nutrient fertilizer for economic reasons.

Water microbial quality in all farms and crop washing facilities was not subjected to monitoring, neither to treatments, although operators used the river water and private wells for the wash. There was a major recognition among farmers of the water pollution in that area. In the summer, when water sources declined, they use private wells for irrigation, whereas in two farms, sewage was used as a source of nutrients to crops, as reported. Unfortunately, they did not perceive the risk of introducing hazards into the produce when polluted water was used for irrigation. Common quotes received from farmers were: "*I know it's polluted with nitrates and other chemicals", "Using Sewage does not harm",* "*lettuce gives higher volume with sewage use*", "*Sewage gives better volume and reduces the use of fertilizers*", "*It doesn't t harm (polluted river), look how big is this lettuce*".

Table 3.2. Characteristics of the agricultural production of selected farms

Farmer	Land production by Dunam (D)	Irrigation source	Adjacent areas	Manure application	Volume of production (Dunam/day)	Transport Time (hrs)	Transport Mode
IB†	Known as biggest producer in Bekaa ND	River	None	Comb‡. Raw-composted manure	1/day (Parsley) 1/2 days (Lettuce) 1/2 days (Radish)	1-3 to Beirut	Open lorry in plastic crates
S	1200	Well/ sprinkles	River	Comb. Raw-composted manure	1/day (Lettuce) 1/4days(Radish) Parsley on demand	2.5 to Beirut	Open lorry in plastic crates
SF 2†	ND§	River/ sprinkles	River	Comb. Raw-composted manure	1/day (Parsley) 1/2 days(Lettuce) 1/2 days(Radish)	1-3 to Beirut	Open lorry in plastic crates
J	90 (850 box Lettuce/1 D) /100 boxes parsley/1D	Sewage/ sprinkles	Landfill, manure storage	Comb. Raw-composted manure	1/day (Parsley) 1/2 days(Lettuce) 1/2 days(Radish)	1.5 to Beirut 3 to North	Open lorry in plastic crates
JF6	ND	Well / river	None		1/day (Parsley) 1/day(Lettuce) 1/3days(Radish)	1-3 to Beirut	Open lorry in plastic crates
RN 1	ND	Well /sprinkles	Chicken farms	Comb. Raw-composted manure	1/day (Parsley) 1/day(Lettuce) 1/3days(Radish)	1-2 to Beirut	Open lorry in plastic crates
SP 7	10,000 basket*24 Parsley /25 days 8,000 basket *6 lettuces	Private well (hesitation during interview)	Landfill, river	Raw	1/day (Parsley) 1/2 days (Lettuce) 1/2 days (Radish)	1-2 to Beirut	Open lorry in plastic crates
SP 10	20	Well/ sprinkles	None	Comb. Raw-composted manure	1/day (Parsley) 1/2 days (Lettuce) 1/2 days (Radish)	2 to Beirut 3 to North	Open lorry in plastic crates
SF 4	ND	Sewage/ sprinkles	Waste collection and manure storage	Comb. Raw-composted manure	On demand	1.5 to Beirut 3 to North	NA
SF 9	40-50	Artificial pond	None		On demand (reserved	N/A	Open lorry in plastic crates

All locations had no traceability system
† Owners of post-harvest washing facilities
§ No data: Undisclosed information
‡Combination of raw and composted manure

3.4.2. The Microbiological quality of fresh produce

Overall, the APC ranged from a geometric mean of 3.50 to 8.39 log CFU/g (Table 3.3), with parsley and radishes having the highest levels (Table 3.4). Two-thirds of the raw vegetables (62%) had APCs above 6 log CFU/g. TC was observed in all vegetable samples, with counts ranging from 1.69 to 8.16 log CFU/g (with 69% having counts ≥5 log CFU/g). *E. coli* was present in almost half (45.5%) of the raw vegetables, with levels ≥2 log CFU/g in more than a third (37%); counts on parsley were significantly higher compared to lettuce and radish. *Staphylococcus* spp. and *S. aureus* were isolated from 91% and 45.5% of all produce types, respectively. In general, the geometric mean *S. aureus* counts was relatively high 4.80 log CFU/g (Table 3.3) and highest for parsley and radishes ($p > 0.05$) (Table 3.4).

Table 3.3. Mean (log CFU/g) [a] of selected parameters of contamination across the different sampling sources, from fields to wholesale market

Microorganism	Source	N	Mean (Range)
S. aureus	F-FP	18	5.50 (3.32-8.39)
	PHW-FP	5	4.51 (3.64-6.38)
	WSM-FP	18	4.18 (< 0.7-6.23)
	Total	**41**	**4.80** (< 0.7-**8.39)**
E. coli	F-FP	35	1.28^{a} (< 0.7-7.00)
	PHW-FP	15	2.24^{b} (< 0.7-6.78)
	WSM-FP	40	2.10 (< 0.7-5.32)
	Total	**90**	**1.80 (< 0.7-7.00)**
TC	F-FP	35	5.13^{a} (1.69-7.60)
	PHW-FP	15	6.04^{b} (5.30-7.60)
	WSM-FP	40	5.92 (3.55-8.16)
	Total	**90**	**5.63 (1.69-8.16)**
APC	F-FP	35	6.52 (3.96-8.39)
	PHW-FP	15	6.23 (5.50-8.27)
	WSM-FP	40	6.39 (3.50-7.88)
	Total	**90**	**6.43 (3.50-8.39)**

F-FP=.Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce, W-WI=Well, water for irrigation, PHW-W= Post-harvest washing water, TC = total coliforms, APC = total plate counts.

[a] minimum detection limit of 0.7 log CFU/g was included in statistical analysis in the event of no visual growth.

Different superscript letters above the means in the same column indicate significant difference at $p < 0.05$.

Table 3.4. Mean (log CFU/g) of selected parameters of contamination in different types of fresh produce

		E. coli	*S. aureus*	TC	APC
	Count(N)	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Lettuce	45	1.71^{a}±1.58	3.85±1.55	5.25^{a}±0.97	6.00^{b}±0.87
Parsley	35	2.17^{a}±1.69	4.69±1.63	6.38^{b}±1.05	6.87^{a}±1.01
Radish	10	0.96^{b}±0.56	4.94±2.10	4.74^{a}±1.59	6.87^{a}±1.01

Different superscript letters above the means in same column indicate significant difference at $p < 0.05$ by ANOVA and Tukey test. For *E. coli,* significance was determined by Games-Howell test assuming non-variance and Kruskal-Wallis test.

3.4.3. Comparative analysis of sanitation and hygienic handling indicators on raw vegetables from the fields to the whole sale market

To identify the critical risk factors along the fresh produce supply chain, a comparative analysis of the bacterial loads on raw vegetables across the interrelated sampling locations was performed. The flow diagram of leafy greens and radish supply chain and identified risk factors is presented in Figure 3.2-4.4.

Farming-Harvesting

- Untrained labourers and farmers on GAP and GHP
- Unsafe water sources for irrigation
- Lack of sanitary conditions and labourers' lodgings on fields
- Unawareness on workers' health and personal hygiene requirements
- Inappropriate handling of manure
- Access of domestic and grazing animals to fields crops and streams
- Proximity to landfills or animal production farms

Some farms rely on sewage directed into irrigation pools or streams

↓

Transportation

↓

Crop washing processes

Figure 3.2. Flow diagram of leafy greens and radish supply chain and identified risk factors from farms to crop washing areas

Crop washing processes

- Lack in monitoring measures and policies to ensure the use of safe water sources
- Shortfalls in washing practices and in maintaining clean water supply
- Basic washing method for a large volume of mixed batches of fresh produce
- Inadequate structural facilities
- Non-sanitized storage implements

Stored in open areas of a washing facility for next consignments

Storage and Packing

Figure 3.3. Flow diagram of leafy greens and radish supply chain and identified risk factors from crop washing areas to storage

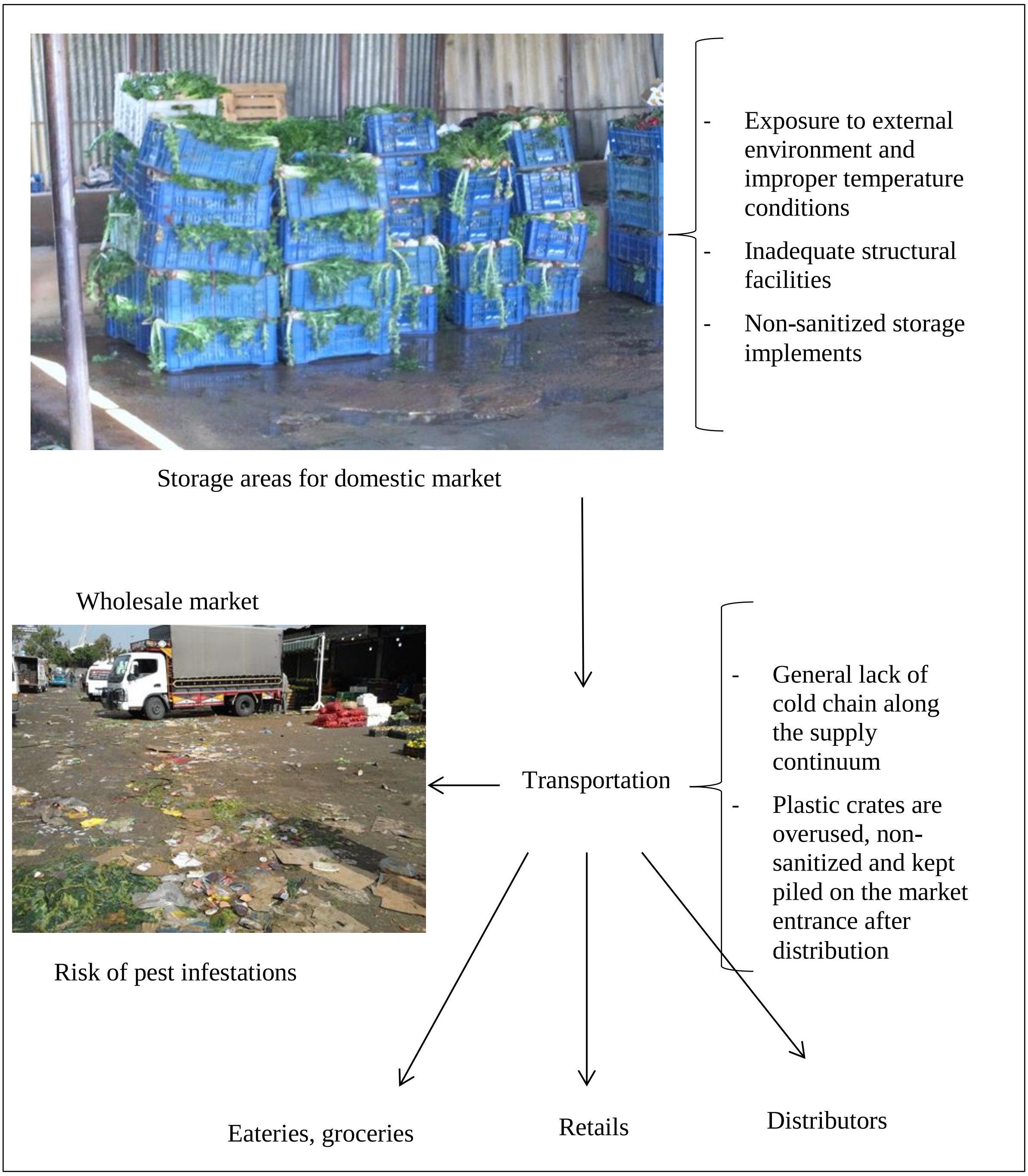


Figure 3.4. Flow diagram of leafy greens and radish supply chain and identified risk factors from storage to retails

The results of the hygienic parameters analysis demonstrated that the population size of APC and *S. aureus* was the highest on produce in the fields, 6.52 log CFU/g and 5.50 log CFU/g, respectively, and that APC almost remained constant throughout the market. The slight decreasing trend of APC levels was apparent from samples taken from farms and at the post-harvest washing stage, while a slight increase was observed thereafter, in the wholesale market. However, *S. aureus* concentration levels on raw vegetables from farms and washing ponds were always higher than on vegetables on display (Table 3.2)

On the contrary, Kruskal-Wallis test showed that *E. coli* mean levels were significantly different across categories of sample sources. Furthermore, Spearman's rho demonstrated a significant correlation between TC and *E. coli* and the sampling sources at $p < 0.05$ and $p < 0.01$, respectively (Figure 3.3). The TC and *E. coli* levels on raw vegetables increased significantly ($p < 0.05$) from the farms (means, 5.13 and 1.28 log CFU/g, respectively) to post-harvest washing and packing for subsequent distribution (means, 6.04 and 2.24 log CFU/g, respectively). Although there was a slight decrease of TC and *E. coli* levels from market samples (means, 5.92 and 2.10 log CFU/g, respectively), these were still higher counts than at harvest.

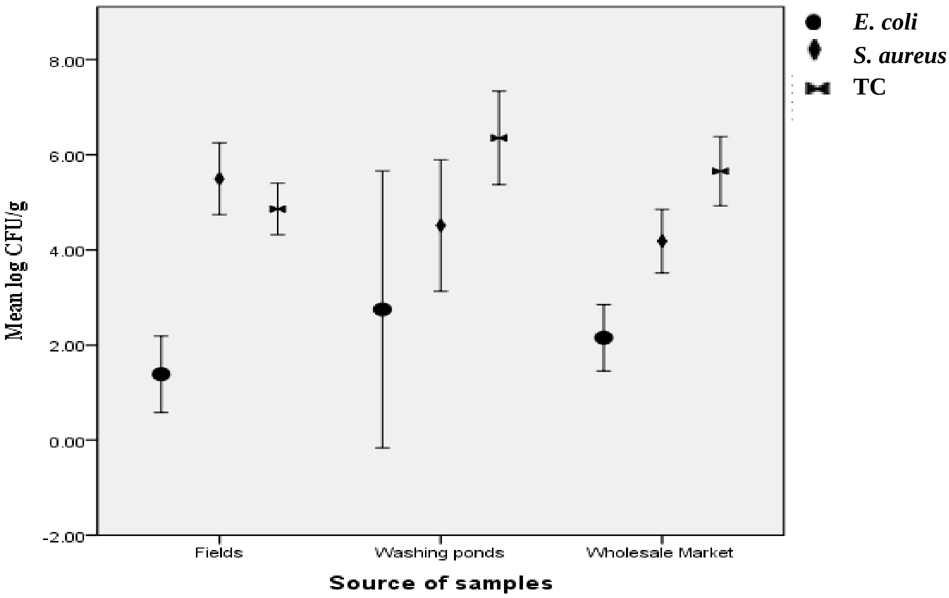


Figure 3.5. Distribution of the mean log CFU/g of *S. aureus, E. coli* and TC on raw vegetables according to sampling sources along the fresh produce supply chain.
Higher values of the mean log CFU/g ±SD in hygiene indicators are demonstrated on fresh produce obtained from the post-harvest washing ponds. Error Bars: 95% CI

3.4.4. Pathogen detection

The prevalence of *L. monocytogenes* was 20% in vegetables in the fields and after washing in the post-harvest areas, but decreased to 8% by the time they reached the retail markets, but in each case at low levels. The overall prevalence of *L. monocytogenes* was 14%. Its prevalence was detected in each sampling location, with equal levels of 20% on vegetables from each, the fields and the post-harvest areas and only about 8% at WSM (Figure 3.6). About half of the ready-to-eat vegetables in the fields (51%) contained *S. aureus.* Although the prevalence decreased slightly (33%) in the PHW stage, it rose again as vegetables reached the WSM (45%). In contrast, the study found only one sample (parsley) out of 15 obtained from the washing pond contaminated with *Salmonella* spp. resulting in an overall prevalence rate of 6.7% for vegetables sampled from the washing area.

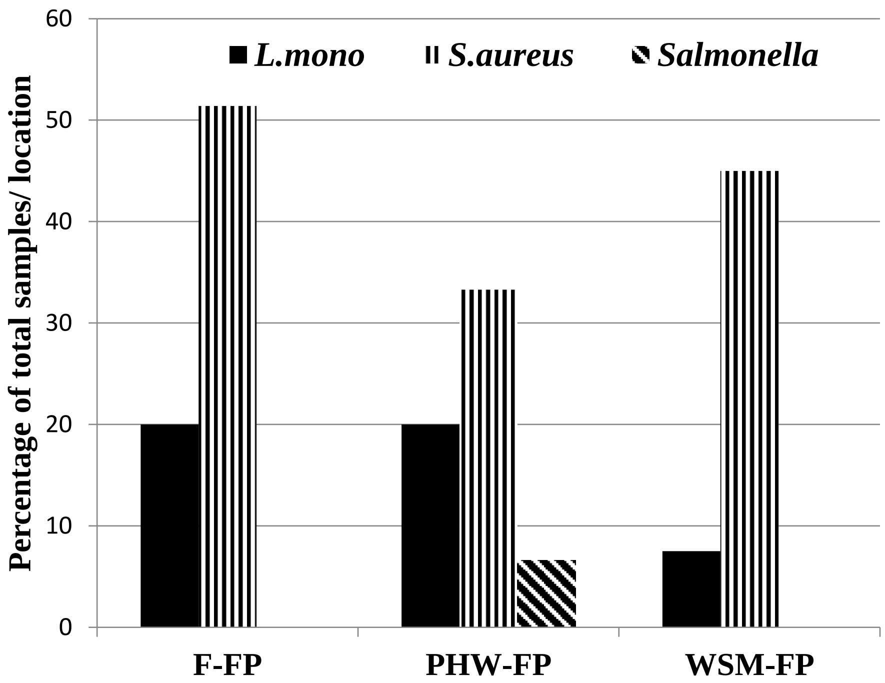


Figure 3.6. The prevalence of pathogens on fresh produce, calculated as the percentage of total samples in each sampling location.
F-FP=Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce.

3.4.5. Microbiological quality of irrigation and wash water

The mean count of *E. coli* in wells water and wash water samples ranged from **<**0.7 - 135 CFU/100 ml and 15-140 CFU/100 ml, respectively (Table 3.5) and the TC was too numerous to count per100 ml analysed samples. Furthermore, water from wells and from river streams used for post-harvest washing and crop irrigation in 5 farms contained unacceptable levels of TC and *E. coli* > 100 cells/ 100 ml, of each. In analysing the impact of water quality used in irrigation on vegetables traced back to its sources, Chi square analysis showed significant association between *E. coli* counts on raw vegetables and the microbial quality of agricultural and wash water. By simple linear regression, a significant regression equation was found ($F(1, 44) = 77{,}174$, $p < 0.001$), with an R^2 of 0.637. *E. coli* counts on fresh produce increased 0.799 for each CFU/100ml of water used. The regression analysis showed that the microbiological quality of agricultural and wash water obtained from same sampling locations of fresh produce is a useful predictor explaining 64% of the *E. coli* variations on raw vegetables that were traced to their sources (Table 3.5).

Table 3.5. The *E. coli* counts on fresh produce in the market traced back to farmers' fields, agricultural water quality and post-harvest washing areas

Farmers	Samples location-type	N	Mean (range)†	Median
Farm IB	F- FP	3	2.80 (<0.70-7.00)	<0.70
	PHW-FP[1,2]	6	1.49 (<0.70-2.88)	0.95
	WSM- FP	11	1.86 (<0.70-5.20)	1.00
	W^-WI	10	36.20 (13.00-80.00)	25.50
Farm S	F- FP	10	1.01 (<0.7-3.84)	0.99
	WSM-FP[2]	13	2.09 (<0.70-4.45)	2.20
	PHW-W	10	83.00 (50.00-140.00)	80.00
Farm B	F- FP	3	3.54 (<0.70-6.00)	4.60
	PHW- FP[2]	3	2.73 (<0.70-6.78)	0.70
	W^-WI	6	50.00 (20.00-135.00)	30.00
Farm J	PHW-FP	6	2.76 (<0.70-4.40)	2.77
	WSM,FP[2]	5	1.32 (<0.70-2.22)	1.30
	PHW-W	10	25.80 (15.00-50.00)	25.00
	W^-WI	10	0.70 (<0.70-6.00)	<0.70

*F-FP=Fields fresh produce, PHW-FP= Post-harvest washing ponds fresh produce, WSM-FP=Wholesale market fresh produce, W-WI=Well, water for irrigation, PHW-W= Post-harvest washing water

† CFU/g for fresh produce samples and *E. coli* cells/100 ml for irrigation and wash water samples

[1,2] *Salmonella* and *L. monocytogenes* detected on produce from this farm

[2] *L. monocytogenes* detected in this farm

3.5. Discussion

3.5.1. On-farm and supply chain fresh produce handling practices

Overall, the lack of control measures to decrease potential contamination of agricultural water, fresh produce and soil from other farm or grazing animals is critical and hazardous as this could possibly expose the fields and water sources to presumptive presence of pathogens which possibly lead to the likelihood of transfer to horticultural crops intended for human consumption, especially when not subjected to a process kill step (CAC/RCP 53, 2003). In some cases, farming fields were located in ca. 2 km away from landfills and chicken farms. Growing produce close to urban areas or land used for other types of agriculture, such as livestock production are reported to be an outcome to increasing populations and land demands, nonetheless this proximity to waste and run-off places high risk to water and fresh produce from potential microbiological contamination. Growing lands nearby urban development and haphazard dumps of slaughter remains in Lebanon is reported by Markou and Stavri (2005), which further indicate the mass of the problem as well as the substantial hazards on consumers' health in the absence of local authorities will to alleviate the problems and reinforce risk assessment in producing edible raw crops. Another critical area is the storage of manure lagoons adjacent to production areas without provisions to prevent leakage or run off. Animal fertilizers used on agricultural land are a well-known source of contamination to vegetable fields (Mukherjee et al., 2007). The use of chicken manure is common and manure lagoons located near or adjacent to production areas were generally not maintained to prevent leaking or overflowing. Chicken manure were bought and stored in land and it treatment was confined to fermentation in only few cases and based on a guess work.

Of particular concern, sprinkle irrigation was typically used as a common mode of irrigation. Research confirmed that sprinkle irrigation compared with furrow and subsurface drip increased the risk microbiological contamination of the edible portion of a crop (Fonseca et al., 2011). In addition to the observed gaps in farming practices, identification of batches to enable traceability and recalls was not performed. In fact, among other requirements for food safety, traceability is generally lacking in the fresh produce supply chain which still faces the challenges of a chronic fragile national food safety system in Lebanon. Interestingly, farmers (SF02 and IB) applied double standards in managing the washing operations for exports compared to the one used for vegetables destined for local use. The agri-food environments call for further investigation of farmers' practices and of impediments to implement proper postharvest practices. Post-harvest washing process is basically taking place nearby the river by dip-washing parsley and radish in ponds filled from the wells at the time of sampling. The source is used without treatments or guidelines defining risk based periodical replenishment. The heavily soiled and turbid wash water was recorded as a result of overuse for all types and incoming batches of produce. On the other hand, lettuces were spray-washed in open crates whilst stacked in trucks prior to distribution to the wholesale market. Generally, the operations took place in unprotected areas where fresh produce was kept in unwashed plastic baskets stored in exposed environment until the next consignment. The methods of transportation of produce to the markets, and handling at the markets required crucial interventions with regard to hygienic conditions and temperature control which appeared deficient at the time of this study. All the observed conditions reflected alarming predisposing factors for microbial contamination of fresh produce via animal manure, contaminated water, domestic animals, and flooding from a nearby contaminated farms or soil (Harapas et al., 2010). Other sources of potential health risk and water contamination were the use of improperly treated chicken manure (Hill et al., 2005).

In most MENA countries, examples of improved infrastructure and management of postharvest handling facilities and technologies (i.e., proper sanitation, temperature control, quality and safety awareness and assurance), along with staff adherence to quality requirements and market regulations were always oriented for export markets (El-Saedy et al., 2011), in contrary to poor examples of handling fresh produce for the domestic market (Figure 3.7). Apparently, there are some common features in agricultural production among MENA countries that lead to losses and compromising the safety of fruit and vegetables from production to consumption and these include lack of education, training and access to good agricultural practices in production. (FAO, 2012)

Whole sale market in Tunisia

Over-Loading of transport vehicles of postharvest in Egypt

Figure 3.7. Storage and transportation of crops. Examples from Tunisia and Egypt (FAO, 2012)

3.5.2. Microbiological survey in relation to identified risk factors

In general, the bacterial loads of the raw salad vegetables exceeded the ICMFS (ICMSF, 1998) acceptable limits of 10^3 to 10^5 (TC) in 100 g of vegetables usually eaten raw.

Moreover, the European Union (2007) established for pre-cut fruit and vegetables (ready-to-eat), a limit value m of 100 *E. coli*/g and a limit value M of 1000 *E. coli* /g. In this context, many of the samples (37%) did not meet acceptable limits for *E. coli*. The overall prevalence level of *E. coli* (45.5%) showed a comparable result to a previous study (42.30%) of vegetables grown in the Bekaa (Halablab et al., 2011). These results are also consistent with a study in Yemen by AL-Jaboobi et al. (2013) which demonstrated that 35% of raw vegetables irrigated with waste water contained *E. coli*. Similar results have been reported in developing countries beyond the MENA Region. Maffei et al. (2013) reported *E. coli* in 40.0% of leafy vegetables harvested in Brazil, and Castro-Rosas et al. (2012) reported faecal coliforms in 99% and *E. coli* in 85% of RTE 130 salad samples originating from vegetables in Mexico irrigated with untreated sewage water. The occurrence level of TC (>5 log CFU/g) in this study (69%) was slightly higher than the prevalence rate reported in Singapore (50%, n=125) (Seow et al., 2012), and it was isolated from all the samples (100%). In contrast, data from western countries reported substantially lower levels of enteric pathogens contamination, such as 8.2% of *E. coli* was recovered from fresh produce in Canada (Bohaychuk et al., 2009), and from only five samples (n=890) in Norway (Johannessen et al., 2002). In the U.S., the range of TC and *E. coli* in leafy greens and herbs, respectively, was <1 - 4.4 log CFU/g and <1 - 1.5 CFU/g, in a study by Johnston et al.(2005). Parsley accounted for the highest overall geometric mean for TC and *E. coli* compared to lettuce and radishes (Table 3.4). The common use of sprinkle irrigation observed in this study, a mode of irrigation frequently linked to increased risk for crop contamination and to higher faecal counts (FDA/CFSAN, 2001; Jung et al., 2014), together with the parsley leaf surface form which could enhance contamination and survival by favouring bacterial attachment and its persistence in curly leaves and crevices (Harapas et al., 2010).

It was surprising to find high levels of *S. aureus* in all the produce items (up to 5 log CFU/g). The contamination level of fresh produce on fields with *S. aureus* did not exhibit a notable change in the post-harvest washing stage. Overall, the high levels showed consistency with some local and international studies (Viswanathan & Kaur, 2001; Halablab et al., 2011), being due to improper handling at harvest (Beuchat, 1995; Viswanathan & Kaur, 2001; Sabbithi et al., 2014). Local environmental conditions could also have contributed to the contamination of the surface vegetables with the survival of *S. aureus* for several weeks (Erkan et al., 2008). Such sources could be from wild or domestic animal faeces, such as sheep pasturing the fields after harvest and before the next seeding, or sewage- polluted irrigation water. However, one major source is inadequately-treated chicken litter which is used as fertilizer by some farms. In this regard, the data concurs with Halablab et al. (2011) who demonstrated that this pathogen was predominant in raw vegetables obtained from areas irrigated with Litani River (51.5%) compared to those in other areas downstream (26.6%). Nevertheless, *S. aureus* might represent public health hazard when growth exceeds 10^5-10^6 CFU/g given optimum conditions or as a result of cross-contamination during handling processes. Similarly, AL-Jaboobi et al. (2013) recorded high counts of *S. aureus* ≥5 log CFU/g on vegetables irrigated with untreated waste water and polluted river water. Interestingly, a recent study in Ghana further highlight the predominance of this bacterial species (50%) on vegetables from cultivated gardens irrigated with waste water and from the market, with mean CFU of around 10^6 CFU/g from each sampling location (Pesewu et al., 2014). More evidently, high level of methicillin-resistant *S aureus* was isolated from the raw sewage of examined treatment plants (Pattillo, 2013) and in the wash water of crops (Ofor et al., 2009). Thus, unlike in studies of vegetables in western countries, *S. aureus* may represent a pathogen of concern that can reach consumers phase in some developing countries.

The variations of microbial population throughout the supply chain were in parallel with previous studies that reported identical levels of APC in the production and retail levels (Ruiz et al., 1987; Johnston et al., 2005; Chau et al., 2014) and the distribution stage (Johnston et al., 2005). There was also a large increase in APC and *Staphylococcus* on carrots as they travelled further through the distribution chain (Ghosh et al., 2004). Although a reduction in bacterial counts could be expected following the washing process, an increase in TC and *E. coli* counts from farms to post-harvest washing was observed, likely originating from the contaminated wash water, based on the observations and consistent with the results of Gagliardi et al. (2003) and Johnston et al. (2005). The high range of *E. coli* levels on washed vegetables (Figure 3.5) is probably because of different water quality experienced during sampling days resulting from inconsistent and unregulated frequencies of wash water replenishments; together with the variable microbial loads of mixed types of produce dipped into the ponds. Therefore, cross-contamination can be explained by transfer from contaminated to clean batches during washing operations in the ponds with no disinfection or sanitization steps (wash-dip for parsley and radishes, or the spray-wash applied on lettuce whilst stacked in open crates on trucks prior to distribution to the wholesale market). This would explain the presence of *Salmonella* on vegetables packed in crop washing areas and the higher levels of TC and *E. coli* on produce at wholesale markets (WSM) than at farms, but compounded by lack of cold chain during transportation and retailing, use of non-sanitized equipment for packing, storage and transportation, and inadequate hygienic conditions at the markets. The results were consistent with Uyttendaele et al. (2014), who maintained that improper hygiene of sellers at open market stalls in Egypt resulted in higher levels of faecal coliforms in produce.

On the other hand, the detection of *L. monocytogenes* on produce from field to the market, also reported by Prazak et al. *(*2002) and Johnston et al. *(*2006). This pathogen has been implicated in listeriosis outbreaks worldwide but not yet in the MENA Region (Todd & Notermans, 2011), and more recently linked to consumption of salad vegetables (Cordano & Jacquet, 2009; Ponniah et al., 2010). The 2011 outbreak of *L. monocytogenes* in cantaloupes with 147 illnesses and 33 deaths in 28 U.S. states, where unhygienic conditions and improper cooling played a role, highlights this risk (McCollum et al., 2013). As it can be found in the agro-environment through shedding by domestic animals (Ivanek et al., 2006; Weiss & Seeliger, 1975), it is not surprising it can also be recovered from river water and ponds used for irrigation, as can *Salmonella* (Combarro et al., 1997; Johnson et al., 1997; Greene et al., 2008). However, there were conditions that would exacerbate contamination. Crop washing operations took place in unprotected open areas, a risky practice as stated by (WHO/FAO, 2008b), and fresh produce was kept in open areas in unwashed plastic baskets until used for the next consignment. The wash water was found turbid from overuse of washing successive batches of produce (replenishment with fresh water supply was based on a subjective visual degree of turbidity). High turbidity levels are often associated with higher levels of pathogenic organisms (U.S.EPA., 2000). Since irrigation and washing of fresh produce can be vectors of pathogenic microorganisms (Solomon et al., 2002; Ibenyassine et al., 2006), water used for post-harvest operations should ideally be potable (Hernandez-Brenes, 2002) and not to exceed 10^3 CFU/ml F.C. /100 ml for the irrigation of raw eaten crops (unrestricted irrigation) (WHO, 1996; Blumenthal et al., 2001; Probst et al., 2012). However, other national and federal guidelines, such as DIN 19650 (German standards), enforce stricter limits considering the water quality is the same as drinking water quality with no *E. coli* or faecal streptococci should be present (Pfleger, 2010) and according to U.S. Environmental Protection Agency and British Columbia, a limit of *E. coli* less than or equal to 77 CFU/100 ml is defined (British Columbia MoE, 2001; U.S.EPA, 2001). It was noted that on one farm wash water ponds derived from the well water with no detectable TC and *E. coli* became contaminated to levels similar to that of nearby river water, indicating that inadequate control allows unacceptable environmental contamination on these farms.

3.6. Conclusion

Based on the literature review, this is the first attempt in Lebanon and the Middle East region to provide baseline information on critical risk factors associated with the microbial quality and on the prevalence of pathogens on fresh produce from the farm to the market. It is apparent that shortfalls in the good agricultural practices (GAP), the lack of clear hygienic guidelines for processing and retailing most likely contributed to the contamination of raw vegetables with *S. aureus* (from chicken litter), TC and *E. coli* and *L. monocytogenes* (from environmental sources). Although *Salmonella* spp. was only found in one sample, an overall prevalence of 1.1% is unacceptable considering the high volume of raw vegetables eaten locally. The crop washing stage showed to be an evident risk area for pathogens transmission to fresh produce and one possible source of crop contamination. The fact that organisms' indicative of faecal contamination was frequently found in levels with the potential for pathogens to be present and surviving on vegetables right up to the consumption stage as raw, should raise concerns (Srikanth & Naik, 2004). Though the knowledge of the precise sources of contamination were not the objective of this study, they are likely the same as have been identified in other regions, e.g., faecal contamination from farms including untreated manure, wild animal reservoirs, human sewage, and infected food workers (European Commission, 2002), especially as it is well-known that the river water used for irrigation and washing is well documented as containing faecal contaminants (Houri & El Jeblawi, 2007) and that cold chain and proper storage and sanitation conditions were largely lacking from farm to the market. Although the current study is not based on representative samples of water and all fruits and vegetables throughout the country or region, the use of contaminated water for irrigation and washing for produce is widespread, and the present results are likely valid for many growing areas in the Middle East. The poor handling practices as well as conditions of transportation and storage facilities of fresh produce in the MENA region is documented, although countries may vary in their standards and enforcement (Kader et al., 2011). There, results on the assessment of crops losses in the region indicated existing lack or poor status of the cold chain infrastructure and basic hygiene along the chain. Consequently, as the developing countries are confronted with stringent requirements of the international market, governments have a pivotal role to set national GAP standards that comply with the recommended requirements of Codex Alimentarius (CAC/RCP, 2003) and to create enabling environment to ensure compliance of stakeholders.

Relevant government authorities should give a high priority to improve and maintain storage and transportation conditions essential for the fresh produce safety and to ensure the implementation of adequate sanitation during the post-harvest washing processes. Equally important, they should enforce an overall water policy in Lebanon (and in other MENA countries) to provide potable water for both urban and agricultural use. In this context, it is advisable that government initiatives and the technical and financial support of international organizations consider the provisions of incentives schemes for farmers who may prefer using nutrient-rich polluted waters to fertilize as well as irrigate crops and are conducive to incorporate strategic solutions for using treated grey water and on-farm wastewater treatment in order to address the economic and water scarcity challenges that jeopardize the safety of the fresh produce

This study emphasizes the application of vigilant sanitation measures, GHP and risk-based preventive measures at retails level to mitigate the risk of bacterial contamination of RTE raw vegetables especially that the immersion process of post-harvest washing was found a point of contamination of fresh produce. Accordingly, it underscores the importance of knowledge in food safety to ensure the application of control measures, and more importantly of raising awareness by informing stakeholders, food handlers and operators and consumers on the associated risks with current practices. Knowledge of food handlers and other factors that likely contribute to their food safety practices and attitudes are hence addressed in the forthcoming chapter.

4. Investigating a link of two different types of food business management to the food safety knowledge, attitudes and practices of food handlers in Beirut, Lebanon

4.1. Introduction

Data on risk factors for food poisoning outbreaks imply that most incidences result from improper food handling practices in food service outlets and homes (Howes et al., 1996) and that contaminated hand contacts during preparation of food are probably the common reason of food handlers' implication in most cases. This is often attributed to employees' lack of knowledge (Greig et al., 2007) and to the poor understanding of food management systems which act as principal barriers against the implementation of basic food safety measures in small and medium-sized food service companies (SMEs) (Ehiri & Morris, 1996b; Fielding et al., 2005).

The provision of food safety and hygiene training and the effective enactment of safe food handling practices are vital to controlling food-borne illnesses. Better food safety information through training and education of food workers, including their certification, has been shown to improve their food handling practices and reduce food contamination during preparation (Lynch et al., 2003; Hislop & Shaw, 2009; McIntyre et al., 2013); however, training alone was not proven the only variable correlated with safe and proper practices on food premises. Knowledge plays an essential role in the enhancement of behaviours and practices, but it is not the only factor that would lead to proper food handling and needs to be complemented with other elements (Seaman & Eves, 2008).

A number of studies used the social cognitive theories to complement the findings on what impedes proper food handling. The Theory of Planned Behaviour (TPB) has been advocated by many researchers to predict determinants of food handler's behaviour (Mullan & Wong, 2010; Seaman & Eves, 2010).

According to the TPB, the performance of behaviour is determined by different motivational factors which work together to influence individuals' behavioural intention (Ajzen, 1991), they include attitude, subjective norm (the pressure perceived about whether or not to perform the behaviour as established by social group identity), and perceived behavioural control (perceived availability of opportunities and resources required to perform the behaviour contributing to the perceived ease or difficulty in its performance). Various studies assessed food handlers' knowledge and attitudes. Whilst several citations assessed the level of knowledge of food handlers on food safety and its influence on attitudes and practices (Baş et al., 2006; Jevšnik et al., 2008; Abdul-Mutalib et al., 2012; Karaman, 2012; Osaili et al., 2013; Abdullah Sani & Siow, 2014), It was shown that perceived behavioural control (PBC) was the most significant predictor of safe food handling intention suggesting that food safety practices are not wholly within food handlers' own control (Mullan & Wong, 2009).

In Lebanon, as in many developing countries, the food safety regulatory framework through the food supply chain is not effectively developed. One reason for this is the antiquated laws responsible for food safety that are not consistent with the international approach that adopts hazard-based and risk-based systems, and overlapping responsibilities of governmental departments and agencies (UNIDO, 2002). This leads to an inadequate enforcement of food law on premises through lack of specific regulations and a limited role for inspections. To date, there is no information on the food safety performance of the food service sector in Lebanon; particularly on the SMEs which share common challenges, such as the lack of resources (time, labour and financial) and lack of technical expertise (WHO, 2000). Furthermore, there have not been significant contributions of scientific studies on investigating the relation of food businesses management with food handlers' attitudes and behaviour towards food safety.

This study aimed to evaluate the level of knowledge, attitudes and practices in food safety of food handlers in SME's in Beirut, Lebanon, and to assess the influential role of two different types of management (developed corporate owned food businesses and less-developed sole proprietor owned food businesses) as a food safety culture element on their perceptions and safe practices in general and in the context of handling fresh vegetables.

4.2 Materials and methods

4.2.1. Data collection and sampling

There are no official data on the overall number of operating food businesses in Beirut. Many small food businesses are operating without a license of registration as reported by a member in the MoT and the warning issued by the Prime Minister's office in Beirut (El Amin, 2013). The mere fact that there was no official data on the overall number of operating FSEs; determination of a representative sample size was not possible. Therefore, a list of 150 FSE formed based on information from internet, MoT and the syndicate of restaurants owners. Besides, some locations were explored accidentally during field work and added to the contact list. Due to the labour and time intensive nature of the data collection, the field work with restaurants was restricted to a convenient sample of 50 food businesses that are located in Beirut and agreed to participate. According to the central limit theorem, a sample size n has to be large (usually $n \geq 30$) if the population from where the sample is taken is non-normal, and if the population follows the normal distribution then the sample size n can be either small or large (Devore, 2011).

The food businesses represented a geographical portion in Beirut and are typical food service outlets in Lebanon and in many countries of the Middle East.

The sampling method involved 2 stage samplings (cluster sampling), first, by concentrating on a geographical area, second sampling respondents (businesses) within those areas The choice on the geographical area was basically affected by 3 factors: i) businesses that are more readily accessible due to limited information on contact address of FSEs in other geographical areas, ii) limited fund and time frame of the funded project to support field trips, iii) the fact that the selected area is famous of being a hub of a high number of restaurants business of all levels and types of cuisines.

The selection of participants was based on:

- The size i.e. micro-small, small, medium. There is still no consensus on a specific definition of SMEs. This is often specific to the country characteristics (APEC, 1994). Several parameters are used to describe SMEs (i.e., number of staff, invested capital, assets value, business volume, and managerial characteristics). In this work, SMEs size was based on the number of employees as per EC criteria (Table 4.1)
- The great majority of enterprises operating in Lebanon could be classified as SME, excluding international chains i.e., McDonalds, KFC, etc.) as these would be expected to follow standards and requirements of global companies.
- Types of food served i.e. raw vegetables salads in addition to other varieties of hot and cold ready to eat foods,
- High number of customers at peak hours, i.e., which can be estimated by observation and number of outlets
- Type of management i.e. sole proprietor and corporate-managed FSEs. By this selection, potential differences in microbiological and hygiene and structural parameters between both types would be investigated. Besides, it would assist in constructing evidence on the supreme role of organizational values in underpinning compliance.

The selection of corporate and sole proprietor businesses was determined based on the business profiles identified during the participation process as well as on professional experience and engagement in the field. The corporate-managed FSEs operate their food outlets in different geographical areas and within the same city through a central management.

Similarly, the traditional or sole proprietor FSEs share common management structure and socio-economic features. In this case, sample selection and size confer a reasonable degree of reliability to this work.

Beirut is a small city where identification of corporate- managed outlets is not complex in view of their market standing, network with food professionals and reputation. Sole proprietor food businesses tend to be informal and lack management structure. They are managed by the owner him/herself, or by head chef with assistants. They are often small bistros, café restaurants, or traditional fast food street outlets, and often known by family names or as time-honoured restaurants.

The international fast food chains and restaurants and supermarkets were not included in this study.

Table 4.1. Classification of SMEs into sizes according to EC criteria

Criterion of SMEs	**Micro**	**Small**	**Medium**
Maximum number of employees	9	49	249
Maximum turnover	≤ € 2 m	≤ € 10 m	≤ € 50 m
Balance sheet total	≤ € 2 m	≤ € 10 m	≤ € 43 m

Source:http://ec.europa.eu/growth/smes/business-friendly-environment/sme-definition/index_en.htm

Restaurants owners/managers were approached by phone to introduce the research objectives and to arrange for an appointment for entry permit to food premises.

In many cases when they were hesitant, an appointment was requested to clarify project details, and then consent forms were also provided and further explained as the opportunity aroused. Some businesses were approached by emails as requested by the managers. It was mainly the case with corporates. The participation process was time consuming taking up to 2-3 weeks of follow up calls and visits to reach either approval or rejection. The rejection rate was 50% for reasons that they are not interested, they have no time, production pressure, lack of trust in inspectors, i.e., suspecting connections with authorities, or simply delayed a decision for too long or failed to answer the phone. Approval for entering premises was the greatest barrier to overcome. It was interesting that most of the contacted corporates were actually more relaxed than entrepreneurs, welcoming the idea; nonetheless, some of the other group also accepted on the basis they needed help to identify the existing gaps in their food business. This survey, including follow up calls and meetings with owners/managers, was carried out over a period of 4 months.

A greater percentage of the participating FSEs (70%) were of micro sized businesses employing less than 10 food handlers, 22% were small sized with 10-15 food handlers, and 8% were medium sized food businesses. The survey, including follow up calls and meetings with owners/managers, was carried out over a period of 4 months.

In our study, the term "food handlers" refers to executive chefs, chefs, assistant chefs, and owners involved in different functions of food handling i.e. receiving, storing, preparing and cooking food.

4.2.2. Development and administration of the questionnaire

A questionnaire consisting of four sections was designed to be administered in a face-to-face interview with food handlers (n=80) to ensure the accuracy of respondents' answers and to avoid external influences. A separate letter of consent for owners and for participant was read explaining the objectives of the research were dually signed by researcher and participants.

Section one: This was designed to obtain demographic information and each food handler's profile such as gender, age, education, working experience, food safety training course attendance.

Section two: This contained 16 multiple-choice questions (each with four or five possible answers), three closed questions and one open question to assess food handlers' knowledge on food poisoning, cross contamination prevention, temperature control, personal hygiene, and sanitation. To avoid chances that food handlers select correct answers and any answer by chance, the multiple-choice answers included “I do not know”. The questions were based on the content of a basic level training courses in food safety and adapted from the work of Burcu Tokuç (2009) and Walker et al. (2003b) with some modifications.

Section three: This aimed at understanding food handlers’ attitudes on a *likert-type scale* that indicates the degree of agreement of respondents to 16 statements on food safety using a three-point rating scale (disagree=1, uncertain=2, agree=3). For this section, the participant was asked about his/her opinion, if he/she agrees with the statements. The score ranged between 0 and 48. The sum of scores was converted to 100 points.

Section four: This demonstrated the frequency of safe handling practices. It included 19 questions on sources of personal hygiene, and temperature control, cross contamination prevention, cleaning, storage and display of food on a five points rating scale (never=1, rarely=2, sometime=3, often=4 and always=5). The score range was standardised between 0 and 100.

The attitudes and practices questionnaires were adapted from the work of Angelillo et al. (2001) with some modifications.

In addition to above categories, additional questions about the process of vegetable preparation including time/temperature and constraints to the implementation of hygienic conditions and safe practices were included (Appendix B).

A pilot study was conducted on seven restaurants, but results were not usable because additional questions were considered and the questionnaire was subjected to few modifications in the section related to practices. It was resubmitted for ethical approval committee in the American University of Beirut and Plymouth University.

After obtaining business owners approval, the project objectives and the consent with cover letter were introduced to explain the purpose of the study, the participant’s rights and confidentiality. Upon approval and signature, face-to-face structured interview was conducted with the participant as conversation-like dialogue to comfort the respondents to speak up and discuss on the topic and may also probe for further explanation.

The questions were clearly read to the respondents in a private setting to avoid discomfort or peer and management influence. The questionnaires were disclosed to management or owner of business from the start to ensure that they know the content and type of information as well as to avoid their possible intrusion during the interview.

The interviews took approximately 45 min. depending on the level of knowledge and education of the interviewees.

4.3. Statistical analysis

All data was analysed using the Windows version of SPSS 21. The data were presented as frequencies, but also averaging of scores on Likert scale was used. It has been used extensively in social sciences and in medical education research considering that parametric tests are sufficiently robust to yield largely unbiased answers when analysing Likert scale responses (Jamieson, 2004; Norman, 2010). Although researchers proposed that attitudes and feelings cannot be measured with the same precision of pure scientific variables, it is generally advocated by social scientists that self-reported data can be regarded as continuous (interval) and used in parametric statistics (Agresti & Finlay, 1997; Pallant, 2007).

The knowledge of food handlers was assessed by scoring a correct answer to each question as equal to 1. The score range was between 0 and 20. The scores were converted to 100 points. A score below 50% of food safety knowledge questionnaire is identified as poor knowledge and 50-70% as limited knowledge considering the percentage of trained respondents. Descriptive statistics were used to summarize the socio-demographic characteristics of respondents and the percent ratio of correct answers. Independent samples *t*-tests were performed to compare selected test parameters between groups. Cross tabulations and chi-square with Fisher's exact tests were used for analysis of associations between tests variables and categorical groups. In some cases, Mann-Whitney U test was used for validation. Results with a p-value < 0.05 were considered statistically significant.

Additional statistics were performed using simple liner regression to test for predicting effect of covariates on the score of the food safety knowledge test. All these factors could have influenced the level of knowledge of food handlers independent of the training. The interaction effect between training and each possible covariate was assessed to rule out the violation of the regression homogeneity assumption. The *F* test result of the product term of training and three possible covariates are as follows:

Table 4.2. Tests of Between-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	8691.442[a]	9	965.716	2.583	.012	.249
Intercept	2319.626	1	2319.626	6.205	.015	.081
Education * Experience	16.436	1	16.436	.044	.835	.001
Education * Position	1308.023	1	1308.023	3.499	.066	.048
Training * Education	392.927	2	196.463	.526	.594	.015
Experience * Position	66.661	1	66.661	.178	.674	.003
Training * Experience	51.915	2	25.957	.069	.933	.002
Training * Position	1236.410	2	618.205	1.654	.199	.045
Error	26169.097	70	373.844			
Total	291247.407	80				
Corrected Total	34860.539	79				

Dependent Variable: Score on test
a. R Squared = .249 (Adjusted R Squared = .153)

Interaction effects were not significant and none violated the assumption of regression homogeneity.

4.4 Results and Discussion

4.4.1. Respondents career background

The majority of the participants were male (93%). This seemed surprising since female food workers constitute a higher proportion in studies in other countries (Jevšnik et al., 2008; Annor, 2011; Abdullah Sani & Siow, 2014; Pichler et al., 2014). This difference might be attributed to different cultures. All of the respondents were involved in the different operational functions from receiving, storing of food as well as in the food preparation and cooking. More than two third of all respondents (70%) fell in the range of 21-40 years of age and the number of head/executive chefs were almost double (38%) the cooks, assistant chefs or section chefs (Chef de Partie). Only 43% of food handlers stated that they had attended basic food hygiene or HACCP courses. One quarter of respondents (25%) attained elementary grade compared with 13.8% illiterate or who attained the primary grade, and to 11.3% and 8.8% of high school and university graduates, respectively. Chi-square analysis showed a significant association of the management environment with the proportion of respondents' education level ($p < 0.006$) and training in food safety ($p < 0.002$). Two third of respondents with hotel management vocational studies (41%) were employed in the corporate-managed FSEs; whereas, the majority of respondents with elementary level (85%) worked for sole proprietor-managed food service outlets and restaurants. The reason is that sole proprietor food businesses recruit more of low-educational levels staff as shown in the data and established in earlier study by Clingman (1977) possibly due to financial limitations.

Similarly, the data showed that two third of food handlers working for corporate-managed restaurants had received training in food safety compared to one third working for individual owners of food outlets.

4.4.2. Overall score of respondents' food safety knowledge

In general, the respondents on the food safety knowledge questionnaire demonstrated a limited awareness in food safety; even though almost half of the respondents were trained on food safety, the average score of correct answers was 56.6 ± 21.0 on 100 score points. The results of this study are comparable to a mean value of 63.2% reported by Jianu and Chiş (2012) in a study in Romania; and similar to a score obtained from 101 food handlers working in a catering institution (56.5%) and 335 food handlers working in nursing homes and kindergartens (60.7%) in Portugal (Martins et al., 2012; Martins et al., 2014). The score was only slightly higher in a Jordanian study by Osaili et al. (2013) who reported 69.4% total score of correct answers (46.47 out of 67 points). Food handlers who attended formal or informal trainings demonstrated higher capability of understanding the questions. Independent samples t-test revealed a significantly higher overall score on food safety knowledge (62.5±21.7) of trained food handlers than untrained food handlers (52.2±19.6). This is consistent with various findings on the empowering impact of training to knowledge (Hislop & Shaw, 2009; Soon et al., 2012; Osaili et al., 2013; Pichler et al., 2014). However, this significance was not established when the percent ratios of answers of trained and untrained food handlers were compared in the area of temperature control and cross contamination (Table 4.4). The experience of food handlers was also proved to significantly affect the level of food safety knowledge (Lynch et al., 2003; Martins et al., 2012); which is as well demonstrated in this study; the total score of food safety knowledge was significantly higher in food handlers who are working for 10 years or more in the food service industry (64.43±18.7) than in food handlers with less years of experience (51.1±20.9), and in respondents who occupy higher positions than cook or section chef. This is similar to results reported by McIntyre et al. (2013) who conclusively proved that supervisory status and years of experience led to improved knowledge scores in both trained and untrained groups.

Interestingly, results indicated significant difference in food safety knowledge scores between food handlers employed by corporate-managed restaurants and by entrepreneurs-managed (sole proprietor) food businesses ($t=2.469$, $df=78$, $p <.016$) (Table 4.3). This is explained by the data that highlighted a higher proportion of trained food handlers and educated respondents in corporate-managed restaurants. Generally, the food safety knowledge of employees working in food handling in Beirut and elsewhere was proved to be inadequate which may translate into unsafe food handling practices.

Table 4.3. Mean scores of food safety knowledge of food handlers grouped by experience, position and type of operation

Groups	N	Mean Score*	Std. Deviation
Previous food safety training			
-Yes	34	62.5[a]	21.7
-No	46	52.2[b]	19.6
Experience			
- ≥10 yrs. experience	33	64.4[a]	18.7
- <10 yrs. experience	47	51.1[b]	20.9
Position			
- Executive/Head Chef	30	63.5[a]	20.0
-Assistant Chef/Cook	44	52.3[b]	21.0
Food service operations			
- Corporate	29	64.0[a]	22.2
- Sole proprietor	51	52.4[b]	19.2

* 100-point scale (n=80)
Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.05$

4.4.2.1. Knowledge on Safe storage, Temperature of food and Danger Zone

Over half (57.5%) of respondents did not neither know what the Danger Zone implies nor the range of temperature that is considered optimum for bacterial multiplication. The significant difference between trained and untrained groups was evident ($p < 0.001$) in this specific area as more than two-thirds of trained food handlers (71%) reported that they knew what the Danger Zone was. However, when respondents were asked to elaborate more by specifying the range, 69% supported their answers wrongly compared to a third (31%) whose identification of the Danger Zone fell in acceptable safe range (between 4°C-6°C and 57°C - 62°C). This is a concern since quotes within the ranges of Danger Zone limits from different source US and UK training materials were allowed. A greater proportion of the latter percentage of respondents was comprised of trained food handlers who tried to recall the precise Danger Zone range learnt from past trainings. Still, this is a remarkably small proportion relative to the number of trained respondents. Food handlers' knowledge related to temperature control and cross contamination has been proved in various studies to be insufficient (Pichler et al., 2014) and was demonstrated to have a lower score than the overall score on food safety knowledge (Baş et al., 2006; Abdullah Sani & Siow, 2014; Martins et al., 2014). Only 11% of respondents in this study identified the correct answer on the hot holding temperature (Figure 4.1); and nearly half of them were trained (44%). This represents a major public health concern since temperature control is regarded as a frequent critical control points in food production and food service. Food handlers often reflected their inability to comprehend the temperature values and its relevance to the degree of heat. The data of this study parallels with the findings of Buccheri et al. (2010) and Abdullah Sani and Siow (2014) who reported that 82% of food handlers did not know the critical temperature of storing hot food.

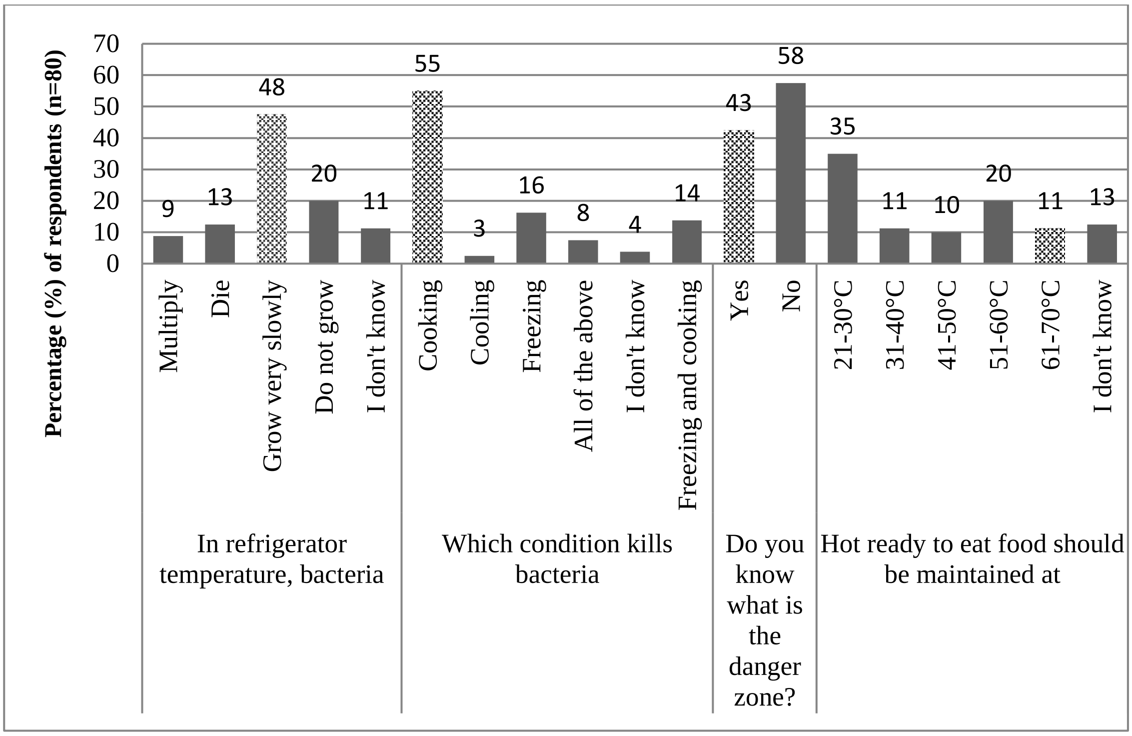


Figure 4.1. The response of food handlers (percentage) to multiple choice questions on the safe storage and temperature of foods.
Pattern- filled column represents correct answers.

The proportion of answers varied greatly when respondents were asked for the correct temperature of cooler and freezer units; 77.5% and 55% of food handlers knew the correct operation temperature of the refrigerator and freezer, respectively. Whereas less than 50% were reported to answer correctly by Osaili et al. (2013) and 69% and 62% by Jianu and Chiş (2012).

Nearly half (48%) were aware of reheating food to ≥74°C, however, untrained respondents (61.5%) were clearly selecting the answer key with highest temperature option (80°C) for the consideration of organoleptic quality of food and customer satisfaction. It is obvious that although trained respondents have significantly higher overall score on food safety knowledge, they demonstrated insufficient knowledge on food temperature requirements. Some were confused by numbers and ranges of temperatures acquired through past trainings, which explains the insignificant difference in the percentage of correct answers when compared to untrained group.

4.4.2.1. Knowledge on contamination and cross-contamination

Approximately half of respondents (49%) identified the correct answer on the source of bacterial food contamination. Half of them did not receive any training. Results are consistent with Martins et al. (2012) who showed a significantly lower knowledge scores on questions related to the control of temperatures, personal hygiene and on the sources of contamination than the overall knowledge on food hygiene. The majority of respondents (95%) knew that raw and cooked food should be separated, but a third of them believe that this is mainly to avoid retention of the flavour/smell of cooked foods from the raw meat or vice versa.

Misperception of respondents for the reason meat should be thawed on the lowest rack of a refrigerator that contain salads was evident; 34% of respondents, nearly half of them (47%) were trained, believed that the lowest shelf has the least cold atmosphere; hence more effective for thawing frozen meat (Table 4.4).

Apparently, improved knowledge on the bacterial hazards along the food chain and on the risks of cross-contamination wasn’t evident among trained respondents; the results parallels a study by Abdullah Sani and Siow (2014) and Walker et al. (2003b) pointed out that this is attributed to the lack of continuous training updates.

Table 4.4. The total percentage of correct answers[1] given by trained and untrained food handlers to questions on contamination and cross-contamination

Question	Answers[d]	Trained[a] N (%)	Untrained[b] N (%)	Total[c] **N (%)**
Where should we place thawing meat in the chiller?	In the lowest rack	24 (52.2)	22 (47.8)	46 (57.5)
Why?	To avoid dripping (cross-contamination)	12 (50.0)	12 (50.0)	24 (48.0)
Where in the cooling unit that contains fresh meats would you store the prepared salads?	On the highest rack in the refrigerator	27 (42.9)	36 (57.1)	63 (79.0)
After using the knife for cutting raw meat, it should be	Thoroughly washed and disinfected	26 (54.2)	22 (45.8)	48 (60.0)
Should raw and cooked foods be separated?	Yes	33 (43.4)	43 (56.6)	76 (95.0)
Why?	To avoid cross-contamination	26 (55.3)	21 (44.7)	47 (61.8)
Which of the following are most likely to cause bacterial contamination	Food handlers, insects and raw materials	19 (48.7)	20 (51.3)	39 (48.8)

[a] The % of trained food handlers of the total % of respondents
[b] The % of untrained food handlers of the total % of respondents
[c] The total % of respondents
[d]The % of correct answers on the sub-question “Why”?
[e] The correct answers to questions on contamination and cross-contamination

4.4.2.3. Knowledge on foodborne disease

In this study, 77.5% of food handlers failed to distinguish between food spoilage and food contaminated with pathogens that leads to illness. They considered that changes in taste, smell and appearance will tell if food is contaminated with foodborne disease bacteria. In a previous study by Walker et al. (2003b) on food handlers working in small businesses, more than half believed they could tell that food may cause poisoning visually or by taste and smell). Over two third of food handlers (70%) have a good knowledge of the most frequent common foodborne disease symptoms; the high proportion of awareness on the symptoms of food poisoning were also indicated in several citations (Jianu & Chiş, 2012; Martins et al., 2012) and in Jordan by Osaili et al. (2013). The most likely reason for this is that foodborne illness is a major health concern and therefore, incidences and symptoms are normally conveyed via media, health campaigns and health practitioners.

4.4.2.4. Knowledge on hygiene and sanitation

The food safety question with the highest percentage of correct answers was related to hand washing frequency. Almost all respondents (90%) expressed awareness on the importance of hand washing after touching raw meat and raw eggs, before handling unwrapped ready to eat foods. The results are similar to findings by Manning (1994) and Soares et al. (2012a) who indicated that 81% and 97.6% of food handlers were aware of the importance of hand washing, respectively. In relation to cleaning and sanitation, two third of food handlers considered that the use of disinfection is the appropriate way to clean knives after use with raw meats. Osaili et al. (2013) reported that 50% of respondents were aware of washing cutting boards and knives used to cut meat or poultry with hot water or hot water and soap before they use them with vegetables.

In general, training of employees was not found to fill gaps in their knowledge in the aforementioned areas which corroborates with the findings of Soares et al. (2012a) and hence, raises concern on one hand on the quality and relevance of delivered trainings and on the other hand on the influence of training intervals on the degree of knowledge retention. Indeed, despite the significant effects of training and other demographic variables on the test score, linear regression analysis confirmed the weak predicting value of training alone to knowledge score, F (1,79) = 4.942, p=0.02 (R^2=0.06). When all predictors variables were entered stepwise, analysis showed that the most fitting and significant regression model would be that combining position, education level and years of experience, and that 19% of the variations in food safety knowledge score was accounted for by those predictors, rather than training as shown in the statistical output below (Table 4.5 A, B, C).

In this context, McIntyre et al. (2013) observed that food handlers' knowledge did decrease over time gradually, when trained under a certified comprehensive programme (FOODSAFE), but the loss was significant when measured over a 15-year span, indicating a need for regular retraining sessions. This finding fits with an earlier study by Capunzo et al. (2005) who also showed an improvement after a food safety training refresher course delivered to food handlers on merchant ships, but this did not carry over to a crew change indicating the need for continuous education to maintain safe practices.

Table 4.5. Regression analysis output

A. Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.307[a]	.094	.083	20.121
2	.383[b]	.146	.124	19.659
3	.439[c]	.193	.161	19.244

a. Predictors: (Constant), Position
b. Predictors: (Constant), Position, Education
c. Predictors: (Constant), Position, Education, Experience

B. ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3281.533	1	3281.533	8.105	.006[b]
	Residual	31579.006	78	404.859		
	Total	34860.539	79			
2	Regression	5102.832	2	2551.416	6.602	.002[c]
	Residual	29757.707	77	386.464		
	Total	34860.539	79			
3	Regression	6714.709	3	2238.236	6.044	.001[d]
	Residual	28145.829	76	370.340		
	Total	34860.539	79			

a. Dependent Variable: Score on test
b. Predictors: (Constant), Position
c. Predictors: (Constant), Position, Education
d. Predictors: (Constant), Position, Education, Experience

C. Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	65.958	3.980		16.573	.000
	Position	-3.414	1.199	-.307	-2.847	.006
2	(Constant)	54.961	6.386		8.607	.000
	Position	-2.999	1.187	-.269	-2.526	.014
	Education	1.780	.820	.232	2.171	.033
3	(Constant)	43.053	8.465		5.086	.000
	Position	-2.501	1.186	-.225	-2.108	.038
	Education	1.846	.803	.240	2.297	.024
	Experience	3.299	1.581	.220	2.086	.040

a. Dependent Variable: Score on test

4.4.3. Respondents' attitudes towards food safety

4.4.3.1. Overall results

The results showed that respondents have a strong agreement on preventive practices (Figure 4.2). The score of food handlers' attitudes was 86.3 ± 13.2 over 100 possible points. All respondents believe that they serve safe food to consumers and that training in food safety and hygiene is essential to their work. A great majority agreed that using cap, masks, protective gloves, and adequate clothing reduces the risk of food contamination (96.2%). These results consistent with studies by Abdullah Sani and Siow (2014) and Buccheri et al. (2010) who reported 93.7% and more than 90% of agreement levels, respectively.

Several studies reported good scores of food handlers' attitudes (Abdul-Mutalib et al., 2012; Soares et al., 2012a; McIntyre et al., 2013; Abdullah Sani & Siow, 2014). However, there were more diverse sets of responses concerning the implementation of temperature control. Approximately one quarter (26.6%) don't consider that measuring the internal temperature of food is important. Furthermore, 22.8% and 16.7% agree that thawing meat on the kitchen counter and keeping dishes containing meat for more than two hours until it cools down at body temperature is a correct practice, respectively.

Various responses were obtained on hands hygiene. Over half (57%) of respondents agreed that they should not touch and work with food when they have cuts and abrasions on fingers. This is in contrast to 37% of respondents who disagreed because of the need to remain on the job due to staff shortages and work pressures. Under these conditions unless properly covered with waterproof bandages, wounds could be infected and then hands become vectors of foodborne pathogens. These figures lead to questioning the measures taken by staff in conditions where hands are vectors of microorganisms to food prepared for consumers and prove that intentions are largely determined by intervening external conditions.

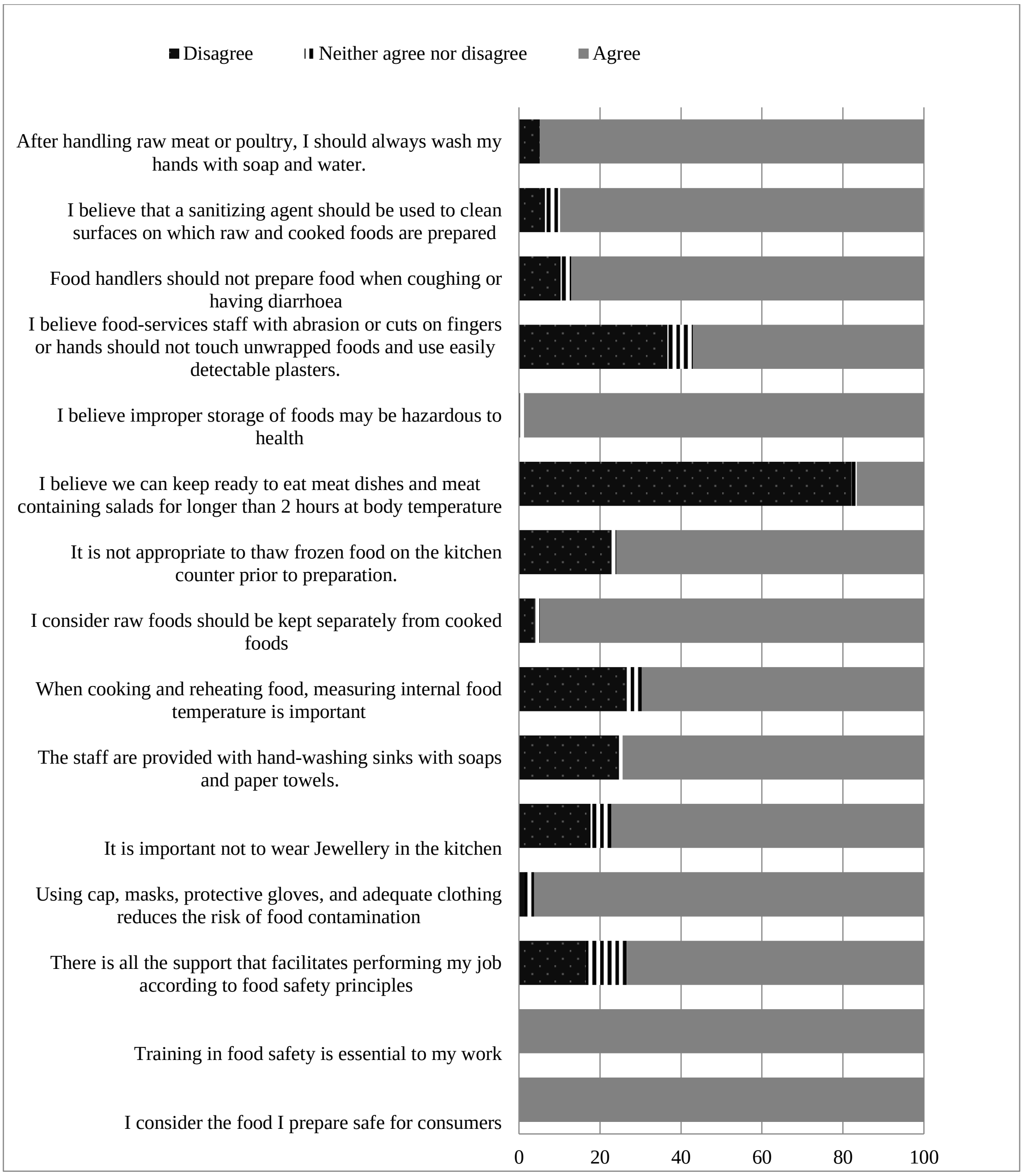


Figure 4.2. The response of food handlers (percentage) to attitude statements related to food safety practices

4.4.3.2. By type of management and training

Chi-square analysis showed that the relation of management to food handlers' attitudes approached significance ($p = 0.056$). Mann-Whitney U test validated this finding and showed that distribution of scores on attitudes are not the same across both the management groups ($p = 0.005$). Findings showed that there is a positive agreement concerning the availability of support that facilitates the implementation of the food safety principles in corporate-managed restaurants (85%) and to a lesser extent in small entrepreneurs-managed food businesses (66%). However, respondents working for small local businesses run by a sole proprietor interpreted “support” as help extended by the head chef who is a role model in that case. In general, the latter group reflected a lack of comprehension of the food safety principles and requirements *per se*.

The analysis showed a significant association of food handlers’ attitudes with the management environment ($p < 0.05$) in relation to the provision of hand washing facility and to the availability of management support; this significance was also noteworthy in relation to statements related to food temperatures (Table 4.9).

A high percentage of food handlers in corporate-managed food businesses (89.7%) compared to (50%) in sole proprietor managed food outlets agreed that measuring internal temperature of food is important. In the latter group, respondents often indicated that they can tell by experience or touch. Hand washing sinks were accessible to the majority of respondents in corporate-managed group (97%) but not to one third of respondents in the other group. Such differences between both groups are expected in view of the financial resources of corporate operated premises, although accessibility to handwashing sink is vital to food safety.

On the other hand, the majority, 93% and 96% of respondents in corporate-managed and sole proprietor-managed businesses respectively, reported that raw food should be separate from cooked food. However, this similarity may not be conclusive evidence on the equivalence of understanding of respondents from both groups in view of the wrong explanation given by half of them when asked for the reason cooked foods should be separated from raw foods (transfer of undesirable odour, taste).

In addition, the results showed no significant difference between trained (87.9±16.5) and untrained group (85.1±10.2) with respect to the overall mean scores on food handlers’ attitudes. The lack of significance may be due to misperceptions of some respondents who believed they served safe food to consumers while control measures and hygienic conditions were not found adequate.

4.4.4. Respondents' food safety practices

4.4.4.1. Overall results

Proper practices and behavior of food handlers during food preparation is crucial for the health of consumers. Overall, results on self-reported food safety practices reflected a limited level of control measures. The score on self-reported practices was 61.3 ± 13.6, with a maximum score of 80 (over 100 possible points).

Safe practices related to temperature control were not properly implemented. More than two third of surveyed food handlers (67.6%) reported that they never measure the temperature of incoming cold or frozen items, further to 75.3% and 70.8% who never measure the food temperature during cooling and reheating as well as during cooking, respectively (Figure 4.3).“*We receive the goods already cold”* was often stated. Besides, the temperature of cooling appliances and food display counters were not monitored by 64.5% and 64.7%, respectively. Respondents often commented :*“we look at the external gauge that display the internal temperature for control”*. The data showed that self-reported practices of respondents were not consistent with their agreement that improper storage of food might lead to health risks.

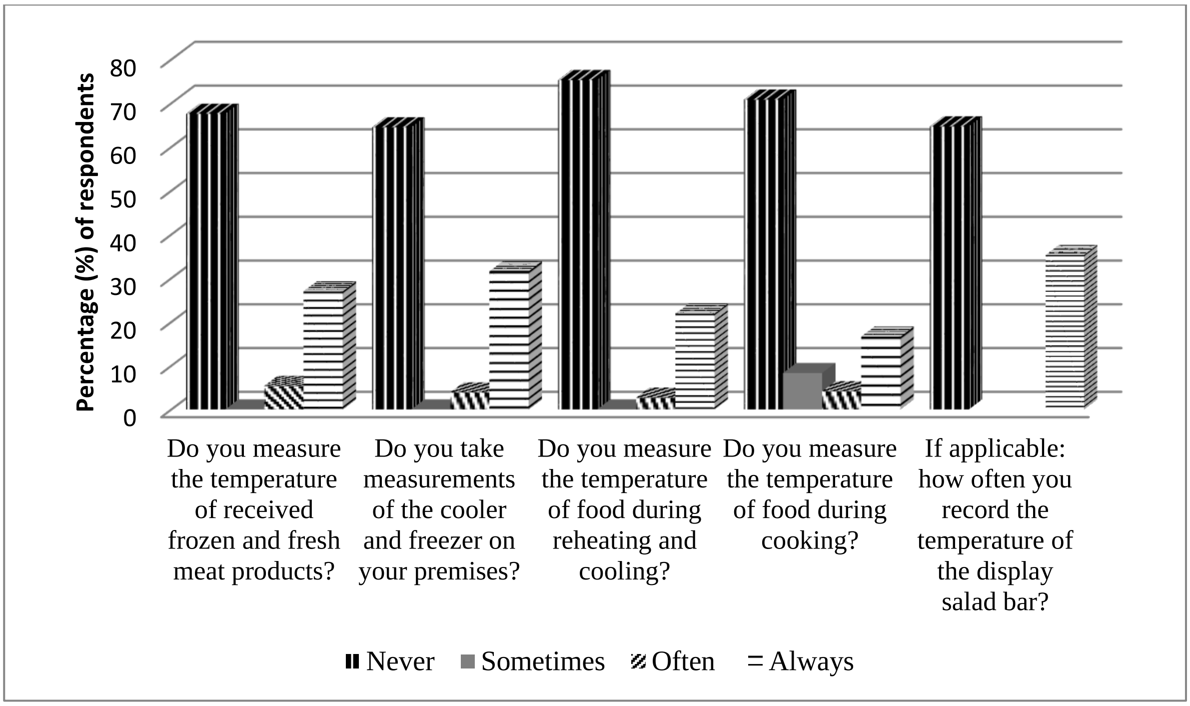


Figure 4.3. Food handlers' self-reported practices related to monitoring of temperatures

4.4.4.2. By management and training

Statistical analysis indicated a significant difference in the practices of food handlers in kitchen in relation to training ($t=3.024$, $df=78$, $p < 0.003$) and management work environment ($t=3.507$, $df=78$, $p < 0.001$). Overall scores of self-reported practices on food safety were significantly higher in corporate-managed group and trained group compared to the overall scores of food handlers in entrepreneurs-managed and untrained group (Table 4.6).

Table 4.6. Mean scores of respondents' self-reported food safety practices

Groups	N	Mean	Std. Deviation
Food service operations			
-Corporate-managed	29	67.9[a]	13.6
-Entrepreneurs-managed	51	57.6[b]	12.2
Training			
-Trained Food Handlers	34	66.4[a]	10.7
-Untrained Food Handlers	46	57.6[b]	14.3

Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.01$

[a] 100-point scale (n=80)

This difference is statistically significant in relation to the mean score on preventive practices relevant to temperature control requirements and sanitation practices (Table 4.8) and substantially reflected as well with the higher trend of disinfection use noted in corporate-managed group (84.6%) compared to entrepreneurs-managed group (39.6%) (Table 4.7).

Table 4.7. The frequency of temperature control and cross-contamination preventive practices by type of management

Self-reported practices	Frequency	Corporate-managed[1]	Sole Proprietor[2]
Do you measure the temperature of received frozen and fresh meat products?	Never	7 (26.9)	43(89.6)
	Rarely	(0.0)	(0.0)
	Sometimes	(0.0)	(0.0)
	Often	3(11.5)	1(2.1)
	Always	16 (61.5)	4(8.3)
Do you take measurements of the cooler and freezer on your premises?	Never	8(29.6)	41(83.7)
	Rarely	(0.0)	(0.0)
	Sometimes	(0.0)	(0.0)
	Often	1(3.7)	2(4.1)
	Always	18(66.7)	6(12.2)
Do you measure the temperature of food during cooking?	Never	8(33.3)	43(89.6)
	Rarely	(0.0)	(0.0)
	Sometimes	4(16.7)	2(4.2)
	Often	2(8.3)	1(2.1)
	Always	10(41.7)	2(4.2)
Do you measure the temperature of food during reheating and cooling?	Never	10(41.7)	45(91.8)
	Rarely	(0.0)	(0.0)
	Sometimes	(0.0)	(0.0)
	Often	(0.0)	2(4.1)
	Always	14(58.3)	2(4.1)
Do you disinfect cutting boards and utensils used on premises?	Never	3(11.5)	27(56.3)
	Rarely	(0.0)	(0.0)
	Sometimes	(0.0)	1(2.1)
	Often	1(3.8)	1(2.1)
	Always	22(84.6)	19(39.6)

[1] The percentage of respondents' answers from corporate-owned food businesses
[2] The percentage of respondents' answers from Sole proprietor-owned food businesses

The TPB holds that the more favourable the intentions and subjective norms, and the greater the perceived control the more likely individual's intention are put into action. Accordingly, the motivating working environment and employees' satisfaction, availability of management's support and resources were proved to be essential elements for the enactment of what have been learnt to ensure safe practices on food premises (Jevšnik et al., 2008; Seaman & Eves, 2010). In this context, this study confirmed that food handling behaviour is not within the person's sole control, it is rather influenced by the type of management that operates the food premises. Well-developed food businesses, comprising structured management with food safety support functions, were found to be directly related to the food safety practices on premises.

Table 4.8. The mean scores of respondents' self-reported practices on temperature control and disinfection is related to the type of employer

Question	Management type	N	Mean score[a]	Std. Deviation	Std. Error Mean
Do you measure the temperature of received frozen and fresh meat products?	**Corporate**	26	3.81[a]	1.8	0.3
	Sole proprietor	48	1.40[b]	1.2	0.2
Do you take measurements of the cooler and freezer on your premises?	**Corporate**	27	3.78[a]	1.8	0.3
	Sole proprietor	49	1.61[b]	1.4	0.2
Do you measure the temperature of food during cooking?	**Corporate**	24	3.25[a]	1.8	0.4
	Sole proprietor	48	1.31[b]	1.0	0.1
Do you measure the temperature of food during reheating and cooling?	**Corporate**	24	3.33[a]	2.0	0.4
	Sole proprietor	49	1.29[b]	1.0	0.1
Do you disinfect cutting boards and utensils used on premises?	**Corporate**	26	4.50[a]	1.3	0.2
	Sole proprietor	48	2.69[b]	1.9	0.3

[a] Mean score on a five-point Likert rating scale
Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.05$

More than two third of untrained food handlers (80%) and nearly all respondents working in sole proprietor-managed food businesses (90%) stated that they never monitor the temperature of received frozen meats products contrary to 45.5% of trained food handlers and 61.5% of respondents working in corporate-managed restaurants who reported frequent "always" monitoring. In spite of this difference, trained food handlers have not reported an appropriate level of safe practices in the kitchen. Only few trained respondents (25.8%) monitor the internal temperature of foods during cooking. A great majority of respondents reported that they use separate cutting boards for raw and cooked foods (89.5%). The use of disinfectants in the kitchen was reported by only half (55%) of the respondents although the majority's opinion obtained in the attitude test was in favor of using sanitizers.

The results on hand washing practices are comparable to those reported by Manning (1994) and Soares et al. (2012a); 80.8% and 89.7% of food handlers stated that they always wash their hands before and after putting on the gloves, respectively.

While the results reflected that corporate-owned enterprises stresses on personal hygiene and offers advantage of resources to support safe practices, preventive procedures, the monitoring tools and systems were lacking overall at the time of this survey. Although the intentions to adhere to safe practices were scored high and were greater in food handlers of this group than their counterpart, safe food handling activities remained inadequate.

In his definition of "Proactive Compliers", a typology of food safety culture which refers to the classification of the different types of food safety culture in food businesses, Wright (2013) stated: "*Management provide a lead in encouraging compliance for sake of the business …but may not go beyond "good practice".* Renowned for their branding strategy to expand locally and/or internationally, the corporate group reveals a proactive type of management. The majority employ food safety officers or third party auditors to run a safe operation, yet they seem to have more critical consideration of their business growth in view of the lack of a comprehensive hazard-based food safety system in place.

Table 4.9. The mean score of food handlers' attitudes on statements related to food safety practices

Statements on food safety related practices	Management	N	Mean[a]	Std. Deviation
Training in food safety is essential to my work	Corporate	29	3.00	0.00
	Sole Proprietor	50	3.00	0.00
There is all the support that facilitates performing my job according to food safety principles	Corporate	29	2.83[a]	0.46
	Sole Proprietor	50	2.42[b]	0.85
Jewelry should not be worn in the kitchen	Corporate	29	2.93[a]	0.37
	Sole Proprietor	50	2.40[b]	0.88
Using cap, masks, protective gloves, and adequate clothing reduces the risk of food contamination	Corporate	29	3.00	0.00
	Sole Proprietor	50	2.92	0.34
The staff is provided with hand-washing sinks with soaps and paper towels	Corporate	29	2.93[a]	0.37
	Sole Proprietor	49	2.24[b]	0.97
When cooking and reheating food, measuring internal food temperature is important	Corporate	29	2.86[a]	0.44
	Sole Proprietor	50	2.18[b]	0.98
Raw foods should be kept separately from cooked foods	Corporate	29	2.86	0.51
	Sole Proprietor	49	2.94	0.31
It is important to know the temperature of the refrigerator to reduce the risk of food safety	Corporate	28	2.96	0.19
	Sole Proprietor	49	3.00	0.00
It is not appropriate to thaw frozen food on the kitchen counter prior to preparation	Corporate	29	2.76[a]	0.63
	Sole Proprietor	50	2.40[b]	0.92
We can keep ready to eat meat dishes and meat containing salads for longer than 2 hours at body temperature	Corporate	28	1.21	0.63
	Sole Proprietor	50	1.42	0.81
Improper storage of foods may be hazardous to health	Corporate	27	2.96	0.19
	Sole Proprietor	50	3.00	0.00
Food-services staff with abrasion or cuts on fingers or hands should not touch unwrapped foods and use easily detectable plasters	Corporate	29	2.38	0.90
	Sole Proprietor	50	2.10	0.97

Food handlers should not prepare food when coughing or having diarrhoea	Corporate	29	2.86	0.51
	Sole Proprietor	49	2.71	0.68
I believe that a sanitizing agent should be used to clean surfaces on which raw and cooked foods are prepared	Corporate	28	2.82	0.55
	Sole Proprietor	50	2.84	0.51
After handling raw meat or poultry, I should always wash my hands with soap and water.	Corporate	28	2.86	0.52
	Sole Proprietor	50	2.92	0.39

[a] Mean score on a three-point Likert rating scale (1=Disagree, 2=neither agree nor disagree, 3 =Agree)
Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.05$

4.5. Conclusion

The results indicated a limited knowledge and common misperceptions of respondents in crucial areas of food safety i.e. temperature control, cross-contamination hazards that could consequently lead to poor and incorrect hygienic practices, the most common causes for food poisoning outbreaks.

It was established that food handlers' attitudes were not consistent with their behaviour and were guided by their food safety misperceptions. Various interfering factors such as misperceptions of "safe and correct practices", the workplace environment and management support influence food handlers' behaviour. Hence, it could well be that respondents carry positive attitudes in every aspect, but they may not have intention to put it into practice. Of particular importance, the self-reported practices were significantly associated with the type of management. In the food businesses operated by sole proprietor or owners, the operational functions remain the responsibility of the owner or the chef. In many cases, they were found to lack interest or awareness in food safety issues, hence food safety is dependent on human behaviour and different external complex factors, i.e., cultural and social background, limited resources and space, lack of skills and a legislative and institutional framework for food safety control. Whereas in corporate-managed food companies with business orientation for branding and franchising, the food safety operations are structured and food safety depends basically on the business priorities of the management and its perception of risks.

The findings of this study confirm the relevance of management type as integral element in the Theory of Planned Behaviour (TPB) for predicting food handlers' attitudes and safe practices. Accordingly, they substantiate future work for assessing traditional food handling practices within the context of the organizational values and perceptions of food safety risks, most importantly within a food safety culture evaluation framework in order to craft effective food safety education and strategy.

This study is the first in Lebanon and among very few in the Middle East region that presents a baseline data for more research on food handlers' behaviours. The outcomes of this study call for national efforts and reform of food safety policies to leverage the role of local authorities in compulsory trainings and inspection routines in view of the overlapping mandate between different governmental agencies. It also underlines the obvious need of food handlers in the SME's for ongoing educational support and technical guidance with emphasis on the key role of cultural and social influences on their perceptions, hence knowledge. Such needs might be best approached by a public and well-established private sector partnership aiming at fostering technical and educational support committees for the SME's.

An observational survey on the food safety climates and food handlers' practices in these food businesses together with microbiological assessment of raw salad vegetables shall determine the reliability of the self-reported practices in this study. More importantly, it will assist to further explore the determinants of safe food handling, and the microbiological indicators linked to identified risk factors and to food safety practices.

5. Empirical approach to assess the food safety climate in different management settings and elements leading to a food safety culture

5.1. Introduction

It is estimated that 50% of outbreaks in 1993-1997 were linked to food consumed in FSEs (Olsen et al., 2000). The typical causes of microbial contamination reported were unsafe source of foods, cross-contamination, poor personnel hygiene practices, inappropriate storage temperatures, and insufficient cooking (Käferstein, 2003; Jones et al., 2008).

Food safety problems are considered to be triggered by food handlers' practices, hence they are thought to be preventable with strategies focusing on education and trainings, which actually proved to be efficient in enhancing the food safety knowledge; however, this was not often the case with improving practices. Knowledgeable food handlers would not necessarily put what they have learnt or acquired into practice (Neal et al., 2012). Several studies reported that even when food workers demonstrate knowledge of safe food preparation practices, they were not always engaged in safe practices (Clayton et al., 2002a; Clayton & Griffith, 2002b). Various constraints preventing applications of food safety requirements were identified. These included the work pressure, limited knowledge, and financial, resources. Understaffing and limited management structure in small restaurants were also identified as major constraints (Fairman, 2004). In this context, implementation of food safety systems is perceived as more of a burden in SMEs than larger companies or food manufacturers (Fairman & Yapp, 2004).

Scientists proposed that an understanding of factors affecting workers' behaviours is necessary to effectively change behaviours (Ehiri & Morris, 1996). The research methods addressed factors beyond internal barriers (knowledge, education), which include the external constraints, e.g. structures, time, resources, and organizational factors (Taylor et al., 2011). Moreover, the applicability of the societal behavioural model also served the understanding of the underlying factors of food handlers' behaviours i.e., TPB (Seaman, et al., 2010). The model is used to obtain data on food handlers' perceptions towards various elements that may influence their intentions and behaviour (Ajzen, 1991). Itis now widely accepted that organizational culture is a vital factor for improvement in food safety practices (Pragle et al., 2007; Fatimah et al., 2014). Lack of organizational support, lack of management and co-workers' encouragement, inadequate facilities and supplies, as well as lack of accountability were identified as barriers related to organizational culture (Green et al., 2007; Pragle et al., 2007; Howells et al., 2008). As such, Clayton et al. (2002) emphasized that behavioural change and practices of food safety will only be realized if a supportive management culture exist, in addition to adequate resources, e.g., structural environment, sufficient staff and time. The term "organization food safety culture", as an element of the organizational culture, emerged from constituents of the safety culture and prevention of healthcare associated outbreaks (Griffith et al, 2010a). It reflects the way the organization applies food safety (Yiannas, 2009), and was recently considered significant as an emerging risk of food borne illnesses in FSEs (Griffith et al., 2010b). Today, there is still no general agreement on the definition of food safety culture. Griffith et al. (2010a) defined the food safety as "*the aggregation of the prevailing, relatively constant, learned, shared attitudes, values and beliefs contributing to the hygiene behaviours used within a particular food handling environment*". The present characterizations of food safety culture are rather based on scientists' in-depth analysis of safety and health studies and of thematic analysis of information generated from food workers. The proposed aspects of the food safety culture were management system and style, leadership, communication, sharing of knowledge and information, accountability, risk perception, and work environment as perceived by food workers (Yiannas, 2009; Griffith et al., 2010a). Poor management commitment, support and communication policy were demonstrated as causes of foodborne illness outbreaks and to a prevailing poor food safety culture (Powell et al., 2011). They are in many cases manifested by the understaffing that could hinder and discourage food handlers from applying proper practices (Green et al, 2007). On the other hand, applications of effective food safety systems or safe operations require that a structured management exist and a control and communication systems are in place in order to verify sound applications. This may be challenging in small sole proprietor food businesses where structured management and delegations of responsibilities is very likely to be lacking. In general, investigations of the culture necessary to influence safe food handling practices is still a recent research venue (Yiannas, 2009; Griffith et al., 2010a). The food safety culture parameters have been generated based on food workers and specialists' perceptions assessed over a set of measurement scales. To date, the actual and most importantly, direct effect of management on food safety standards and practices have not been observed or empirically explored. The translation of management commitment onsite can theoretically vary with workers' perceptions and understanding of management role in food safety. Therefore, the objective of this study was to conduct an in-depth observational assessment of the food production environment and practices in food operations operated by two extreme types of management (sole proprietor and corporate-managed operations) in order to gain insights into elements that are necessary for a food safety culture in FSEs and generate valid measures of the contributing role of management on hygiene and food safety standards. This comparison shall shed light on what may withhold well-developed FSEs management to adopt comprehensive preventive measures for foodborne illnesses and on key determinants of a food safety culture. As very little is known on how management types impact handling processes of fresh salads vegetables in SMEs, this study also aimed at evaluating the contribution of management types in reducing risks associated with handling fresh ready-to-eat salads vegetables (RTEs).

5.2. Material and Methods

5.2.1. Data collection and sampling

The selection of sole proprietor and corporate managed SMEs was based on the sample selection process described in chapter 4. An in-depth observation study of SMEs (n=50) located in Beirut was conducted to assess hygiene standards and handling practices of food handlers during the salad vegetable preparation.

The observation survey was performed by one researcher (myself) which helped to ensure consistency in data collection. The survey involved observations of the overall cleaning and hygiene conditions, structural and environment conditions on premises and handling practices during the preparation of fresh salad vegetables starting from receiving until serving.

5.2.2. Survey design

The survey checklist comprised essential components in which the GHP and other prerequisites proposed by the Codex Alimentarius (1969) were included for the general assessment criteria. It covered all areas including documentation and recordkeeping requirements which are crucial parts of a food safety system (CAC, 2010). Additional components in relation to salad preparation practices and to evidence for systems monitoring and records were also included (Appendix E). The criteria for each component were defined to specify limits for classification (Appendix F).

The checklist focused basically on 6 constructs of 2- 7 components for analysis (Table 5.1).

Table 5.1. The six different constructs comprised in the visual assessment survey in SMEs

Inspection constructs	Individual Inspection Components
Construct 1: Structural compliance	a. General maintenance conditions and evidence of pest in the production environment b. Zoning (separation of fresh produce from raw meat and poultry) c. All major pieces of equipment such fridges, freezers ovens, hot holding equipment, cold holding equipment are fitted with working temperature monitoring gauges d. Availability of proper handwashing sink
Construct 2: Personal hygiene	a. Wearing hair cap b. Appropriately clean personnel protective clothing
Construct 3: Sanitation	a. Clean floors, walls, overall facilities and implements b. Waste containers are covered, kept clean c. Sanitizers for work surfaces readily available for use during food preparation d. Containers used to drain vegetables are kept clean
Construct 4: Evidence of procedures and management system control	a. Records keeping for verification of temperature monitoring and system audits (during cooking, cooling, storing) b. Cleaning system and schedule c. Where a chemical sanitizer is used, there are records to show levels are maintained
Construct 5: Contamination and cross-contamination control measures	a. Staff cleaning tools are stored in appropriate manner and not at risk of contaminating food or equipment during preparation b. Staff personal belongings are stored in appropriate manner and not at risk of contaminating food or equipment during preparation? c. Received fresh vegetable are stored in protected areas d. Washing sink designated for fresh produce only e. Unprocessed raw vegetables are prepared so that contamination and cross- contamination does not occur (separate cutting boards and utensils) f. Visitors or unauthorized staff are granted protective clothing upon entry g. Entry for authorized personnel only
Construct 6: Safe and hygienic handling practices	a. Appropriate use of gloves and handwashing b. Frozen food is properly thawed c. Vegetable sanitizers are made up correctly d. Food on hold is covered

A reliability analysis test was performed to measure the internal consistency in the survey questionnaire. Cronbach’s Alpha was 0.928 which indicates a high level of internal consistency for the scale.

Table 5.2. Reliability Statistics

Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	N of Items
0.928	0.932	26

Additional 8 questions on handling practices of fresh vegetables during receiving, washing and storage were posed to food handlers (n=80) via the face-to-face interviews conducted in chapter 4. The questions were ranked on a five points rating scale (never = 1, rarely = 2, sometime = 3, often = 4 and always =5).

To ensure consistency and unbiased data records, the data collection and visual assessment were carried out by myself.

To avoid or reduce the Hawthorne effect, thus the false interpretation of positive and adequate practices (Haas & Larson, 2007), the observation checklist was not directly exposed in order to avoid food handlers discomfort. Purpose of the visit and the non-affiliation to any official bureau were confirmed. Observations were abbreviated during the inspection process concomitantly with smooth friendly interactive discussions during the inspection of premises and salads preparation. One food handler was observed at a time in each location in the course of salads vegetables preparation.

Components that were either “not observed” or “not applicable” were not included in the statistical comparisons or tabulations, hence omitted from scoring.

5.3. Statistical analysis

All data was analysed using the IBM SPSS version 22. Data was collected and grouped according to food service management type, i.e., sole proprietor or corporate-managed food businesses.

Observational assessment of each component was rated based on three units' scale (adequate=3, incomplete=2, inadequate=1) for 26 components. For statistical representations, the sum of the total awarded units on adequacy level (total score) for each sampling location ranged between 26 and 78 units and was converted to 100 points.

Frequency of rating on adequacy level in each component was obtained and Independent t-test was also used to determine differences in total score on visual assessment of all components between corporate-managed and sole proprietor-managed FSEs. The frequency of food businesses in each adequacy level for each category was calculated.

Spearman's rho correlation test was performed to examine strength of association between types of management and scores on the visual assessment of overall components.

For further understanding at the level of each single component, Chi-square cross tabulations Fisher exact tests in addition to Somers'd tests, an ordinal measure of association appropriate to distinguish between a dependent and independent variable, was used to understand the association pattern between types of management operating food production and the adequacy level of conditions and handling practices.

Logistic regression was performed to test the extent management can be an explanatory or predictor to total inspection score.

5.4. Results

5.4.1. General hygiene conditions and safe practices

In general, the t-test revealed a statistically significant difference between both types of management in relation to their overall visual assessment score across all components of hygienic conditions and practices on premises (t=5.914, df=48, $p < 0.001$). Premises operated by corporates reflected better commitment to hygienic conditions and practices and had a higher mean score in the overall visual assessment (77.88±18.45) than food businesses operated by sole proprietor (48.47±12.82) (Table 5.3).

Table 5.3. Mean value of inspection scores on the visual assessment of overall components in SMEs adequacy level of food service establishments

Locations (by management type, Overall)	N	Mean* ± SD
Management type		
Company	12	$77.88^{a} \pm 18.45$
Individual/Family	38	$48.47^{b} \pm 12.83$
Overall locations	50	55.53 ± 19.01

* over possible 100 points
Different superscript letters in the same column denote statistically significant differences at $p < 0.05$
t=6,206, df=48, $p < 0.05$, with a mean difference of 29.411

More specifically, the mean scores on adequacy level for each of the six different constructs assessed during the observation were significantly higher for premises managed by corporates than those managed by sole proprietors in relation to structural conditions of premises (t=7.068, df=37, $p < 0.001$), cleanliness and sanitation (t=5.912, df=37, $p < 0.001$) and cross-contamination preventive measures (t=5.865, df=26.533, $p < 0.001$) (Figure 5.1) and for individual component levels (Table 5.4); there was significant difference in the mean of scores across the personal hygiene indicators indicating greater commitment observed in corporate-managed locations in terms of personal hygiene protective clothing (t-3.635, df=25.924, $p < 0.001$) and wearing hair cap (t=4.294, df=48, p<.001), and correct use of gloves during salad vegetables handling (t=4.756, df=15.042, $p < 0.001$).

Table 5.4. Mean value of adequacy level in practices related to cross-contamination, safe handling and sanitization by type of management

Conditions and practices	Management	N	Mean ± SD
Food handlers wearing gloves correctly and appropriately	Corporate	12	$2.42^{a} \pm 0.79$
	Sole proprietor	38	$1.24^{b} \pm 0.59$
Floors, work surfaces, utensils and equipment are kept clean	Corporate	12	$3.00^{a} \pm 0.00$
	Sole proprietor	38	$2.13^{b} \pm 0.90$
Correct use of cutting boards and utensils to prevent cross contamination	Corporate	12	$2.83^{a} \pm 0.58$
	Sole proprietor	38	$1.58^{b} \pm 0.82$
Premises structural conditions	Corporate	12	$3.00^{a} \pm 0.00$
	Sole proprietor	38	$1.92^{b} \pm 0.09$
Sanitizers use for work surfaces	Corporate	12	$2.83^{a} \pm 0.57$
	Sole proprietor	38	$1.55^{b} \pm 1.06$

Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.05$

However, despite marked differences between both groups, the performance level in terms of sanitation and cross contamination preventive measures over-passed the internal monitoring and control in corporate group as reflected by the lack of evidence of records. Recording and monitoring the temperature of foods during holding, cooling and cooking were not adequately performed in both groups. Food handlers in the sole proprietor group relied on the external digital thermometer display of cooling appliances or their own experience by touching and feeling to tell if foods were properly cooled or hot. About one third (37%) and an additional 16% did not have properly functioning temperature monitoring gauges or internally fitted thermometer in all or in at least one of their cooling appliances, respectively, which was predominantly observed in sole proprietor group (Figure 5.1).

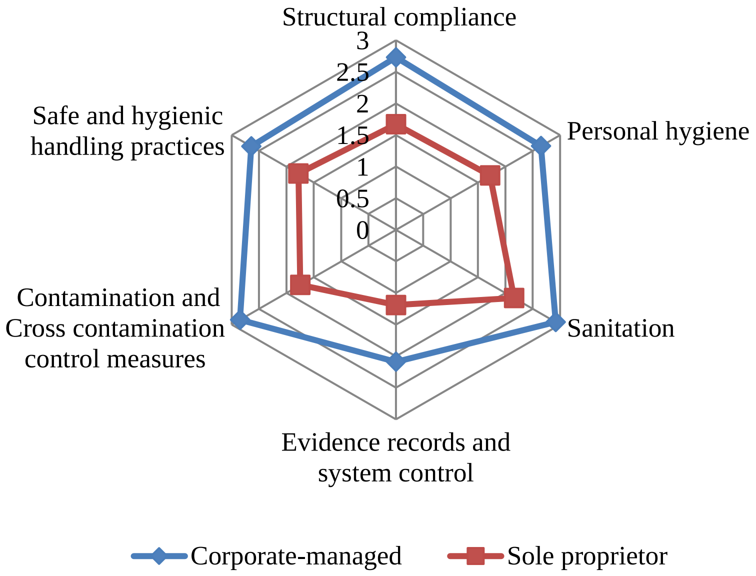


Figure 5.1. Average score by type of management on a 3-points scale with 3 signifying the highest compliance in the hygiene standards and handling practices

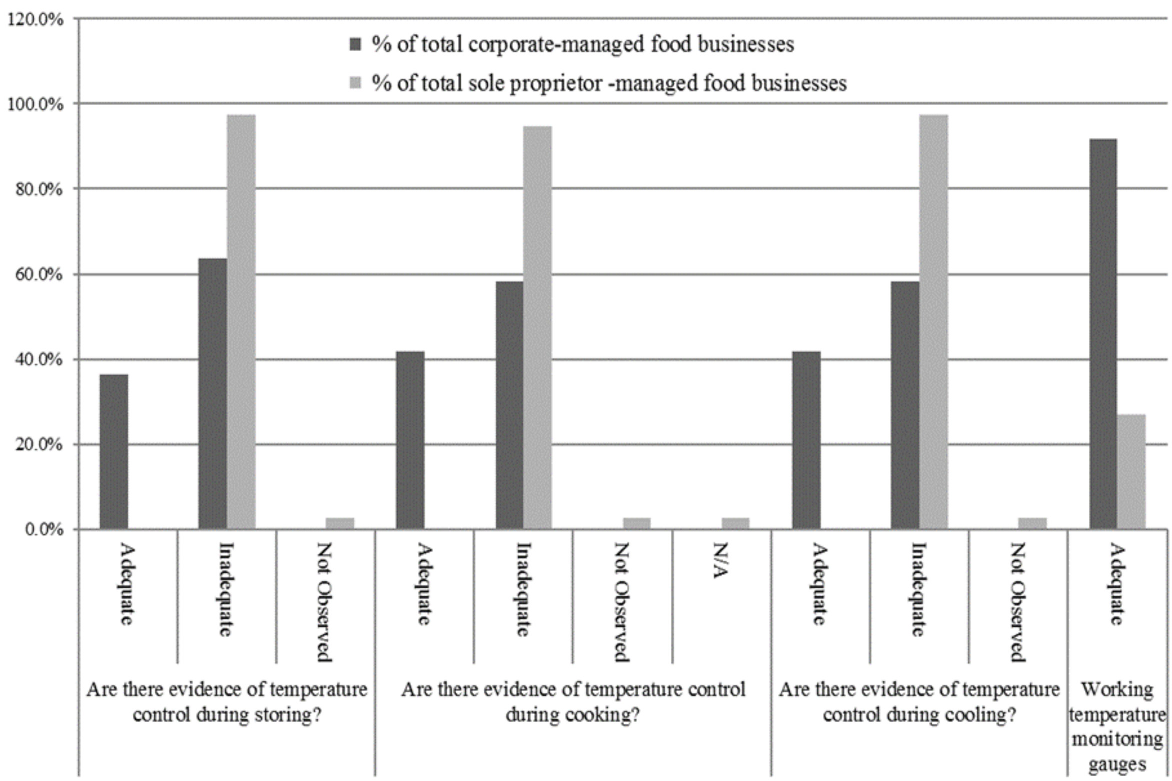


Figure 5.2. Distribution of adequacy level in temperature monitoring according to management types. A fraction of 2.5% of practices related to auditing records in sole proprietor businesses were not observed**.**

As a result of limited working spaces commonly observed in sole proprietor locations, various risk factors inside food preparation premises were observed. A large proportion of sole proprietor restaurants (71%) did not have separate areas for personal clothing and shoes of food handlers as well as for cleaning tools which were often observed in food production areas on shelves nearby implements or food ingredients. In addition, high-risk and low-risk risk foods and appetizers were prepared at the same time in a very small area that hardly fit a handwashing sink in 65.8% and 8% of sole proprietor and corporate-managed businesses, respectively (Table 5.5).

Chi-square and Fisher's exact test showed a significant association between types of management and the adequacy level of compliance. More specifically, there was a significant association between the types of management and adequacy level of premises, e.g., well maintained walls, drains, protection against pest entry, and measures taken to ensure separate preparation of raw and cooked foods ($p < 0.001$). This was also found in relation to adequate use of washing sinks designated for fresh fruits and vegetables and of sanitizers use for contact surfaces and implements on premises at a value of 22.934 and 25.812 ($p< 0.001$), respectively. Cramers'v and Phi tests values indicated generally strong relationships (0.67-0.75). In parallel to Chi-square analysis, Somer's d test also indicated a strong association between assessment components and type of management which was statistically significant. Somer'd coefficient ranged between 0.52-0.78 ($p < 0.05$) for all components with exception to components related to temperature monitoring and record systems and use of sanitizers (0.18-0.36) (Table 5.6 and Table 5.7). Accordingly, it was shown that more than 50%-78% on the adequacy level on the different constructs are explained by management type. Additionally, Spearman's rho correlation indicated a statistically significant association between management and overall adequacy score ($r_s=0.571$, $p < 0.001$).

Table 5.5. The distribution of adequacy level in hygienic and safe practices by type of management

Observation components	Inspection Rating	**% of total corporate-managed food businesses (n= 12)**	**% of total sole proprietor - managed food businesses (n=38)**
Zoning and space	Adequate	41.3%	13.2%
There are hand-washing facilities in food handling areas supplied with warm soap and disposable towels	Adequate Not Observed	75.0% 0.0%	5.3% 10.5%
The cleaning schedule is visible	Adequate Not Observed	33.3% 33.3%	0.0% 13.2%
Sanitisers for work surfaces are readily available for use during food preparation	Adequate Not Observed	91.7% 0.0%	10.5% 10.5%
Floors, work surfaces, utensils and equipment are clean	Adequate	100.0%	39.5%
Waste containers are covered, kept clean	Adequate	91.7%	29.7%
Food handlers use gloves appropriately and correctly	Adequate	58.3%	7.9%
Unprocessed raw vegetables prepared so that contamination and cross contamination does not occur	Adequate	91.7%	13.2%
The received fresh produce are stored in protected areas	Adequate Not Observed	91.7% 8.3%	31.6% 15.8%
There is a washing sink designated for fresh fruits and vegetables only	Adequate	75.0%	7.9%

Table 5.6. Statistical output of Somer's d association test of inspection components with management types

	Visual assessment components†													
	1a	1b	1c	1d	2a	2b	3a	3b	3c	4b	5c	5d	5e	5f
Somer's d Coefficient	0.66	0.49	0.58	0.65	0.67	0.52	0.55	0.61	0.61	0.56	0.41	0.78	0.71	0.74

† Inspection components (dependent) were measured in relation to independent variable "Type of management" and coefficients showed stronger association with components related to general hygiene practices, cleanliness, staff personal hygiene and well maintained facilities

Table 5.7. Measures of weak association of components rated by visual assessment with the type of management operating food service establishments

	Visual assessment components				
	4a cooking	4a cooling	4a storing	5g	6c
Somer's d Coefficient	0.35	0.32	0.38	0.18	0.26
Approx. sign	0.007	0.007	0.02	0.036	0.008

In line with previous statistical tests, the regression analysis showed that management could statistically and significantly predict the total inspection score, F (148) = 38,510, $p < 0.001$ and accounted for 44.5% of the explained variability in overall score (Table 5.8).

Table 5.8. Model Summary

A

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.667[a]	0.445	0.434	14.313

[a] Predictors: (Constant), Management

B

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	7888.826	1	7888.826	38.510	.000[b]
	Residual	9832.887	48	204.852		
	Total	17721.713	49			

[a]. Dependent Variable: Score
[b]. Predictors: (Constant), Management

C

Model		Unstandardized Coefficients B	Std. Error	Standardized Coefficients Beta	T	Sig.	95.0% Confidence Interval for B Lower Bound	Upper Bound
1	(Constant)	107.296	8.583		12.500	0.000	90.037	124.554
	Management	-29.411	4.739	-0.667	-6.206	0.000	-38.940	-19.882

In comparison to self-reported practices, the observational assessment showed inconsistency and disparity in handling practices across different indicators related to personal hygiene, safe handling of food and risk control measures when compared to self-reported practices in same facilities (Figure 5.3). Self-reported practices concerning compliance to the use of protective clothing and gloves, the use of separate cutting boards for raw meat and vegetables, and the application of disinfections as well as storing of fresh vegetables in protected areas were not consistent with the results obtained during the simultaneous observation of the same respondents on the same day of the interviews. There was a great discrepancy between those who reported that they wore protective gloves to prevent cross-contamination and those very few who were actually observed performing crucial tasks wearing the gloves. The frequency level of essential practices for ensuring safe food production was reported by food handlers in 36 to a maximum of 42 surveyed food service businesses. In contrast, respondents did not show and translated what they reported in practice. Correct practices were visually assessed as “adequate” in only 10 to a maximum of 20 inspected locations (Figure 5.3).

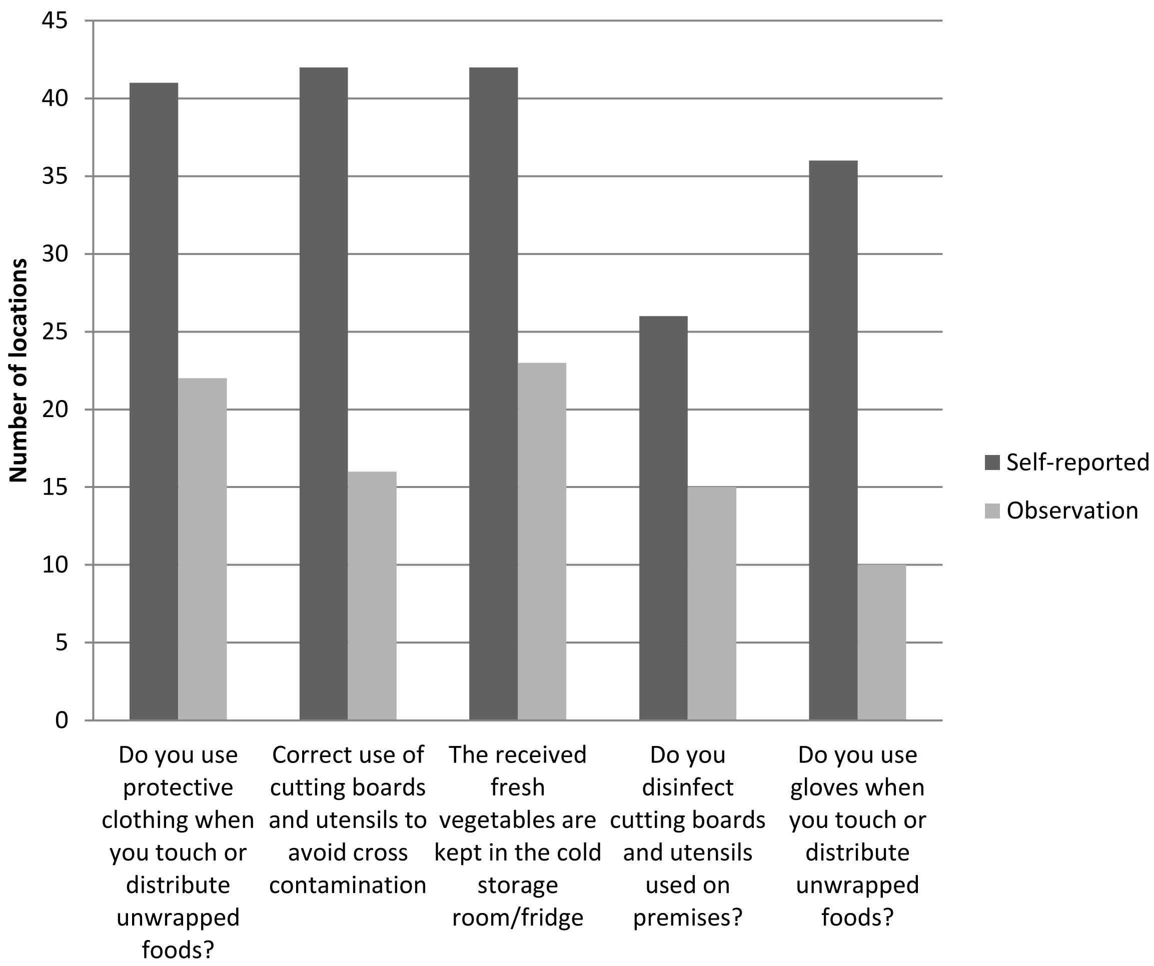


Figure 5.3. An illustrative chart on the proportion of self-reported food handlers' practices as frequently performed i.e. "always" in comparison to the proportion collected via observational survey.

5.4.2. Handling practices of salads vegetables preparation

A large proportion of FSEs (84%) didn't have their water treated with chlorine or filtered, particularly in small sole proprietor businesses. Whereas, it was noted that corporate-managed restaurants had automated system for wash water disinfection which was a reason they didn't chlorinate their water supply tank and rather used filters. Correct dilutions of sanitizers and vinegar (when used) was noted in 75% of corporate group compared to 23% of sole proprietor businesses (Figure 5.4).

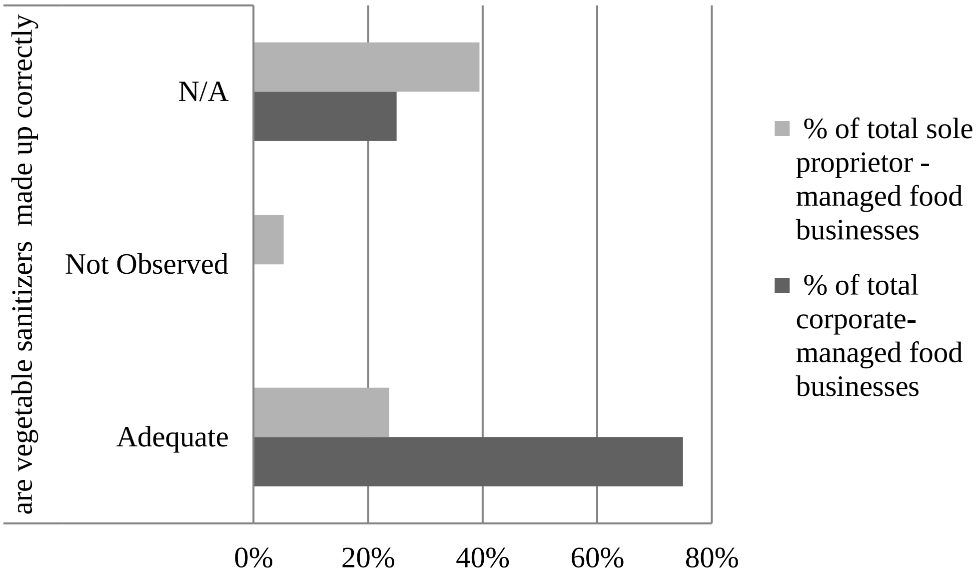


Figure 5.4. Distribution of adequacy level in proper use of vegetables sanitizers according to management types.
N/A=not applicable

The majority of respondents reported that fresh produce is stored in cold storage. They recorded a mean value of 4.63 and 5.00 in the sole proprietor and corporate group, respectively (Table 5.9), whereas inappropriate storage of fresh vegetables was observed in 38% of the premises; of those that showed adequate storage in properly clean and cooled store rooms only a third was in sole proprietor group where fresh produce was seen placed on floor at kitchen entrances, in external open areas or counters until use due to limited storage capacity and space. Overall, the latter group scored significantly lower than corporate group on self-reported handling practices of fresh vegetables with regard to treatment of wash water and temperature control of salad bar/displayed RTE salad items (Table 5.9).

Table 5.9. Mean value of adequacy level for handling practices of fresh vegetables

	Management	N	Mean* ± SD
- Is the washing water used for fresh vegetables and fruits chlorinated?	Corporate	27	$2.33^{a} \pm 1.922$
	Sole proprietor	50	$1.32^{b} \pm 1.096$
- Do you wash the vegetables before cutting?	Corporate	21	4.43± 1.434
	Sole proprietor	45	4.04± 1.678
- The received fresh vegetables are kept in the cold storage room/fridge	Corporate	24	5.00± 0.00
	Sole proprietor	48	4.63± 1.142
- The washed and cut vegetables for salads and garnishes are held at room temperature before preparation/service	Corporate	20	$1.00^{a} \pm 0.00$
	Sole proprietor	46	$2.57^{b} \pm 0.00$

*Mean scores on a 5 points rating scale with 5.00 denoting full compliance "always"
Different superscript letters above the means in the same column indicate significant difference within groups at $p < 0.05$

5.4.3. Perceived Barriers

The interview with food handlers identified a number of barriers impacting their ability to implement the basic food safety requirements (Figure 5.5).

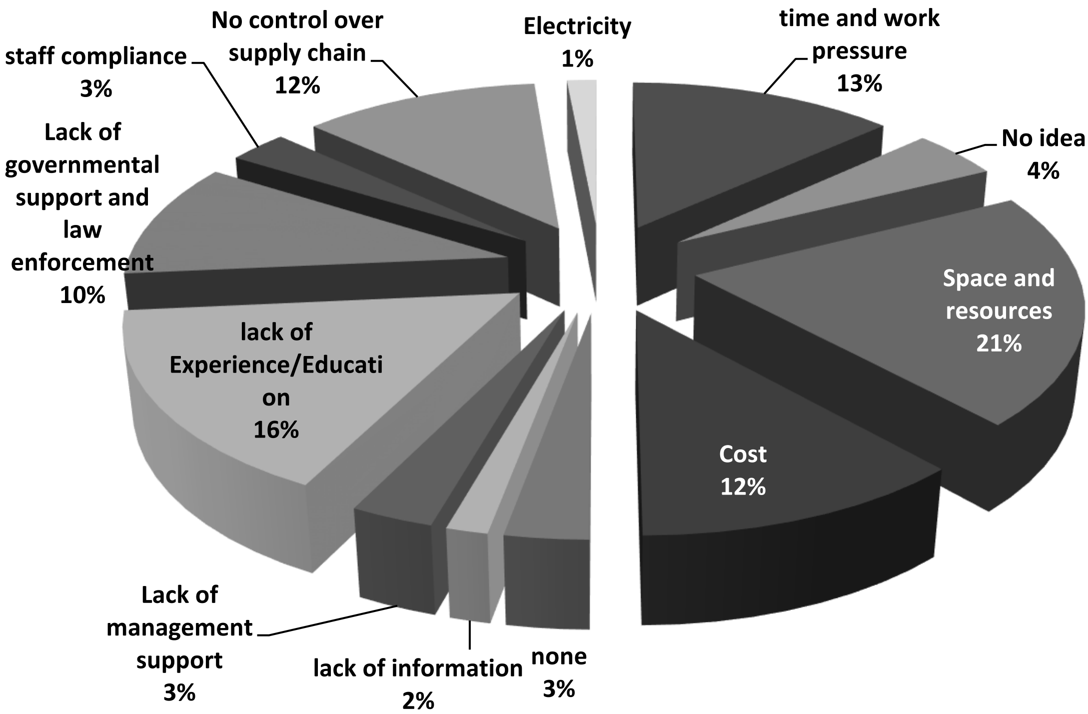


Figure 5.5. The challenges that food handlers perceived as barriers against the implementation of a food safety system or safe practices during food preparation

Many respondents (21%) expressed discouragements due to lack of space and limited in resources. In addition, 13% of respondents considered that time and work pressure especially in peak hours of food production are hurdles to proper food safety actions. Equally important, was the understaffing, cost for maintenance, equipment and essential tools for monitoring temperatures. The 12% of the respondents believe that the lack of financial back up and support by owners bring about limitations for the improvement of the work environment and structure. It also affects the ability of staff to handle their tasks efficiently as a result of understaffing; Access to know-how and finding ways to get food safety tips and guiding procedures were main concerns for 16% of respondents working for sole proprietor FSE. They considered that information resources and guidance for the understanding and implementation of procedures were not available. Guidance is needed to attain adequate hygienic conditions and practices.

This study highlighted additional elements related to the inefficient role of local health authorities' inspectors, which drive 10% of respondents in sole proprietor group to criticize the system and not to comply with safe practices. For them, the health inspectors issued reports with no subsequent follow-ups or guidance for corrective measures. In addition, 12% spoke of the deficits in the food safety control throughout the food supply chain, thus the application of food safety preventive measures on premises are not necessary. For instance, it was stated that:" The *system is lacking across the supply chain and it already predisposes our own raw materials to unavoidable hazards."*

5.4. Discussion

It was obvious that self-reported practices of food handlers did not parallel their actual practices during the visual assessment, particularly in relation to personal hygiene practices and temperature control. The inconsistent translation of food handlers' affirmative opinions towards food safety into actual practices is documented (Oteri & Ekanem, 1989; Manning, 1994; Neal et al., 2012). Additionally, Bermudez-Millan et al. (2004) demonstrated through household observations that claims of food safety behaviours related to hand washing and sanitation were not necessarily put in practice. The observation tool was verified as an effective instrument in determining the food safety behaviour confirming as well the limitations of using self-reported practices to come up with valid scientific statements. There were several barriers that prevented food handlers from applying safe behaviours. These were in line with various studies that indicated lack of time, training, and resources as barriers to hand washing, thermometer use and cleaning of work surfaces (Clayton et al., 2002a; Green et al., 2006; Howells et al., 2008), besides the inconveniently located hand sinks and lack of space (Howells et al., 2008).

The sole proprietor group demonstrated insufficient knowledge and resources to support food safety training programs or to allow staff to attend off-site training, e.g., some food handlers spoke of understaffing and stressful environment. Others for instance mentioned that they don’t know where to get information on food safety from.

Besides the prevalent poor knowledge among the food handlers and more critically, the lack of handwashing sinks, the sole proprietor group was characterized with an informal management of a limited communication structure, lack of specialized food safety and quality department, and understaffing. Food handlers often expressed resentment from the work environment conditions and the fact that they are handling multiple tasks at a time. Considering the lack of handwashing sinks and work pressure in many of sole proprietor SMEs, food handlers would not be expected to put into action what they otherwise reported in chapter 4. The workforce was composed largely of employees with no background in food safety and low educational level as described in chapter 4. The chef is usually in charge for the food production (entrusted to the business) or the owner. In few cases, the business owners consulted the opinion of the chef for the participation in this research.

The results reflected an unawareness and limited understanding of the importance of cross-contamination and contamination preventive measures in small sole proprietor-managed FSEs. Owners of the sole proprietor restaurants were entrepreneurs who showed no interest in this subject and its significance to health during the participation process.

Conversely, the corporates group demonstrated a superior hygiene conditions and supportive environment for food production. Most of the challenges encountered in the other group were surmounted by the corporate group. The latter demonstrated superior food safety environment compared to sole proprietor, manifested in the infrastructure, equipment provisions, well-structured departmental functions of food operations and safety, communication system (as noted during the participation and communication process), adequate operational and supervisory staffing and delegations of authorities and functions that basically steer effective communication of management decisions with food handlers. This group has a greater capacity for proper management of food and hygiene operations in view of higher proportion of educated and trained food handlers (chapter 4). It is typically operated by well-structured management with a well-known brand image and market standing as local branches and in some cases local and international franchises. The results paralleled with Clayton and Griffith (2008) who emphasized the management role in instilling a culture of food safety. The FDA (2011a) proposed that supervisory function is key for ensuring improved food safety practices and that the manifestation of an effective management control through active engagement in implementation of the food safety practices and fostering supervisory control functions are regarded pivotal for maintaining safe practices.

The difference in practices and overall hygiene conditions between both groups can be also explained by the high exposure of FSEs with renowned and marked eateries to the market and to attention of health inspectors. In this case, they are driven to maintain clients trust and expectations as well as to ensure appropriate structural and hygiene operations

Although the corporates were obviously adept to extend resources needed to maintain acceptable standards in hygiene, the observations underlined gaps that reflected the limited management involvement in food safety management, Apparently, there was a concentration on gearing resources towards the pre-requisites for food safety systems rather than risk preventive processes. It is good to hint back on the example of the lack of thermometers for monitoring foods and storage temperature in almost overall the locations surveyed, although a great majority of respondents agreed on the importance of controlling the temperature and on the management support in food safety. The comparative onsite observations validated that management commitment and support functions can be translated in various ways. It is also perceived differently according to workers’ attitudes and knowledge level as described in chapter 4.

It is assumed that reasons other than financial factors interfered with the management decision. This is more likely attributed to leaders/decision makers’ unawareness of the risks associated with to food safety. During the interviews, the food safety officer for one of the corporate-managed locations pointed out on the top management’s emphasis for a clean production facility, personnel hygiene, and on the cleanliness of the customers’ seating areas than on reinforcing control measures of food poisoning during food preparation. An absence of a risk-based national food control system and risks communication can adversely limit consumers and stakeholders’ awareness on food safety. This is also related to the local authorities’ role in bringing up incentives and benefits for the food service industry to adopt robust food safety systems rationalized for implementation without additional burdens.

5.5. Conclusion

It was substantiated that management type had a direct impact on personnel hygiene and overall hygiene level on premises. Despite better adherence to safe practices of food handlers in corporate-managed sites compared to their counterpart group, safe food handling activities remained incomplete vis-à-vis implementations of preventive measures to reduce foodborne illnesses. The observations suggested that food safety should be rooted primarily in the corporates/owners' values as a critical issue that constitute the basis for successful management systems and active management engagement in order to attain a strong food safety culture and safer food handling.

Accordingly, this study shed light on the significance of evaluating the attitudes, drivers and food safety values of management leaders/decision and their relationship to compliance to food safety standards in future research work. Furthermore, it underlined the need for necessary improvements in sanitary and hygienic practices in sole proprietor SMEs to minimize microbial hazards on RTE raw vegetables. Further investigation was undertaken in the successive chapter to assert the impact of management types and the food safety climate on the microbiological hazards in fresh vegetables.

6. Microbiological quality of ready-to-eat fresh vegetables and their link to food safety environment and handling practices in restaurants

6.1. Introduction

Fresh vegetables are rich sources of water-soluble vitamins and other nutrients essential to improve the nutritional status and decrease the risk of cardiovascular disease (Su & Arab, 2006). However, when they are not carefully prepared, they can be subjected to pathogenic contamination and become hazardous to health particularly when eaten raw (FAO/WHO, 2008).

Outbreak investigations often indicate that FSE greatly contribute to foodborne illnesses involving fresh produce (Jones & Angulo, 2006; Sodha et al., 2011). Multiple studies revealed that food workers were frequently engaged in unsafe food handling (Manning, 1994; Clayton & Griffith, 2004; Sneed, Strohbehn, & Gilmore, 2004; Rajagopal & Strohbehn, 2013) and that microbial contamination of RTE foods typically occurred in FSEs with food handlers as asymptomatic carriers of pathogenic microorganisms or with poor personal hygiene being involved (McEvoy et al., 2004; Todd et al., 2008). Equipment or surfaces that have not been effectively cleaned or remained wet between cleaning and use also serve as direct routes for contamination of ready to eat foods (Evans et al., 2004; Gill et al., 2001), besides inappropriate storage temperatures, and insufficient cooking (Jones et al., 2008; WHO, 2007).

Less information is available on the relative health risks attributed to handling practices and preparation procedures of raw salad vegetables in SMEs, while other RTE foods and meats have attracted more attention.

Inspection tools are essential for capturing information on the general hygiene standards and food handlers' practices Although private or local authorities' inspections are an effective mechanism to assure compliance to food safety standards, there is no a clear indication of a correlation between risk of foodborne illnesses and inspection scores. There have been many cases when restaurants scored high on inspections and were still having critical violation in food safety (Jones et al., 2004). The significance of association of microbiological quality of RTE vegetables to hygiene inspection scores has not been fully investigated and not sufficiently addressed by researchers. Earlier attempts to establish direct relationship between the results on microbiological analysis of food and visual inspections have not been successful and were mostly based on foods of animal origins (Wyatt & Guy, 1980; Tebbutt & Southwell, 1989; Powell & Attwell, 1995).

The objective of this study was to analyse the prevalence of pathogens and microbial contamination in salads vegetables and food contact surfaces of 50 food service facilities by regular sampling during handling processes from the receiving stage until display and service. It also aimed at investigating risk factors that may be associated with the microbial safety of fresh produce in SMEs. Such investigation will provide further insights to potential links of microbiological safety of fresh produce with handling practices in order devise effective food safety interventions.

6.2. Material and methods

6.2.1. Observational survey

A sample of fifty SMEs located in Beirut were observationally assessed for hygiene standards and handling practices of food handlers during the salad vegetable preparation. The survey checklist is described in Section 5.2.and presented in Appendix E and Appendix F.

6.2.2. Additional information

Additional 8 questions on handling practices of fresh vegetables during receiving, washing and storage were posed to food handlers (n=80) via face-to-face interviews and were ranked on a five points rating scale (never = 1, rarely = 2, sometime = 3, often = 4 and always =5).

6.2.3. Sample collection

6.2.3.1. Management of samples

A total of 118 samples of various fresh-cut RTE salad vegetables (lettuce, parsley, arugula, coriander, cucumber, tomato and radish) prepared in fifty restaurants were collected after washing and cutting/chopping. On average, three to four types of vegetables were sampled from each restaurant, being subjected to availability and preparation plans at times of visits. They were placed in a sterile bag by food handlers at the end of the preparation process by means of utensils or tools typically used when bringing them into display or storage containers, taking care that they would not touch the inside of the bags.

6.2.3.2. Swabs of cutting boards and knives

Before cutting/chopping vegetables, surfaces of cleaned cutting boards and knives (normally cleaned by assigned cleaners in well-established restaurants, or food workers in less developed restaurants) were swabbed by moistened cotton-tip in BPW (Bio-rad laboratories Ltd, Hemel Hempstead, UK) in three different directions: left to right, top to bottom, and diagonal over a 50 cm^2 area for cutting boards and a length of ca. 10cm on knives. The swabs were placed in tubes of 5 ml BPW for subsequent analysis within 30 min.

6.2.3.3. Microbiological analysis of samples

Samples of salad vegetables were analysed for the presence of pathogens and hygiene indicators organisms commonly isolated from RTE fresh vegetables, i.e., *S. aureus*, *Salmonella* spp., *Listeria* spp., *L. monocytogenes*, in addition to APC, *E. coli* and TCs (Sagoo et al., 2001; Nguz et al., 2005). TCs are considered as a useful indicator in this study for the overall GHP and conditions in which pathogens are generally present in lower counts (FDA, 2002), rather than indicators for poor temperature control considering that vegetables were sampled instantly after the receiving and preparation processes. Levels of faecal organisms such as *E. coli*, are generally considered useful hygiene indicators of the general sanitary conditions in the processing environments, and more indicative of faecal contamination reflecting potential presence of enteric pathogen such as *Salmonella* and conditions that can support their growth (Nguyen-the & Carlin, 1994; NACMF, 1999)

The microbiological analysis was performed as described in Sections 3.2.2.1 and 3.2.2.2. The counts were reported as means of colony-forming units (CFU) per g and were converted into log CFU/g.

Additionally, for statistical purposes, *Listeria* spp were ranked into three levels (above 100 CFU/g, below 100 CFU/g, and not detected).

6.2.3.4. Swab tests

The swabs in 5 ml tube of BPW were vortexed vigorously for 1 min. Tenfold serial dilutions were spread-plated onto duplicate plates of PCA, RAPID'*Staph* agar supplemented with egg yolk and RAPID'*E. coli* 2 agar (Sneed et al., 2004). Counts were expressed as log CFU/swabbed area.

6.3. Statistical analysis

All data was analysed using the IBM SPSS version 22. Observational assessment of each of the 26 components was rated on three units' scale (adequate=3, incomplete=2, inadequate=1). The sum of the total awarded units on adequacy level (visual assessment scores) was converted to 100 points.

Frequency of levels in compliance (adequacy level) for each visually inspected component was obtained. The differences in bacterial levels among different compliance levels were compared using One-way ANOVA, and independent t-test was performed to compare results between two groups.

The association between bacterial counts and overall visual assessment scores was assessed by Pearson correlation and multiple linear regression analysis; binomial regression was performed for *S. aureus*.

The percentage variances in bacterial counts (log CFU/g) explained by individual inspection components were determined by correlation ratio ETA^2 (η^2 ratio). In the case *Listeria* and *S. aureus*, Spearman's rho and cross-tabulations Somer'd tests were also applied.

6.4. Results

6.4.1. Overall results on food handlers 'practices and hygiene conditions on premises

Results of the visual inspections of FSEs and food handlers' practices during the preparation of fresh salads vegetables indicated structural inadequacies and insufficient fulfilment of hygiene prerequisites with a mean score on overall adequacy level of 55.5 ± 19.0 over 100 possible points (Figure 6.1), with the majority of locations being below scores of 50-70.

Over half (54%) of the food premises failed to fulfil the basic hygienic requirements for clean floors, equipment and food contact surfaces, while a third had limitations in the structural conditions (Figure 6.2). Recorded incompliances included open drains, gaps and holes on windows and walls and evidence of pests (cockroaches) at the time of the survey. Furthermore, 22% had not a completely well maintained premise, i.e., no gaps and holes, in good repair, no peeling paints. More than a half (52%) of the FSEs had space limitations compromising the preparation of food safely, whereas only 22% of premises had taken measures to separate areas for the preparation of raw meats and RTE foods. It was notable that the inappropriate sanitation measures were not applied in 60% of the premises (Figure 6.2). Only 8% of FSEs had cleaning schedules, and showed evidence of temperature monitoring records of salads display and cold storage.

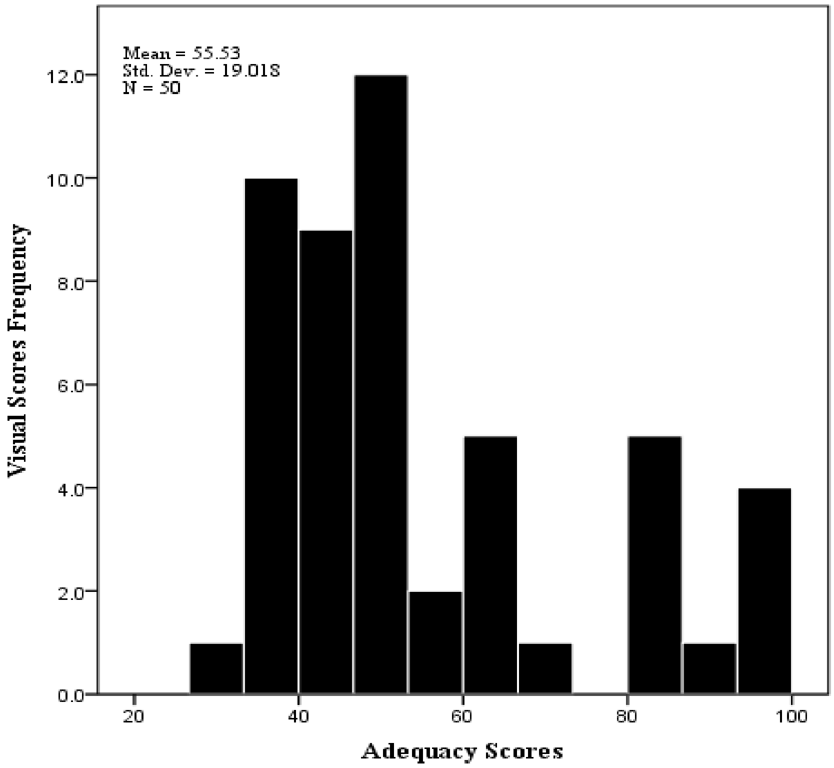


Figure 6.1. The distribution of total score obtained from the overall visual assessment of hygiene conditions and handling practices

In addition, a large percentage of food businesses (64%) lacked hand washing sinks; or designated sinks for washing fresh fruits and vegetables were either absent (32%) or if fitted, it was not clean and was also used for others purposes such as washing hands or implements used with raw meat and cooked foods (40%). More concerning, gloves were used correctly and appropriately during the salad preparation in just a fifth (20%) of the premises. Risks of cross-contamination were detected in 48% of the premises, for example by the presence of heavily chipped or unclean cutting boards, unfamiliarity of food handlers with the concept of color-coding or separate use of utensils and cutting boards for raw meat and fresh vegetables. There was misuse of colour-coded cutting boards in 18% of FSE's where colour-coded cutting boards were used for several types of food. The component "frozen foods are thawed properly" was not observed in 74% of the premises visited, yet it was inadequately performed in 14% of the locations where frozen fish or chicken soaked in water were noted at the time of the visit.

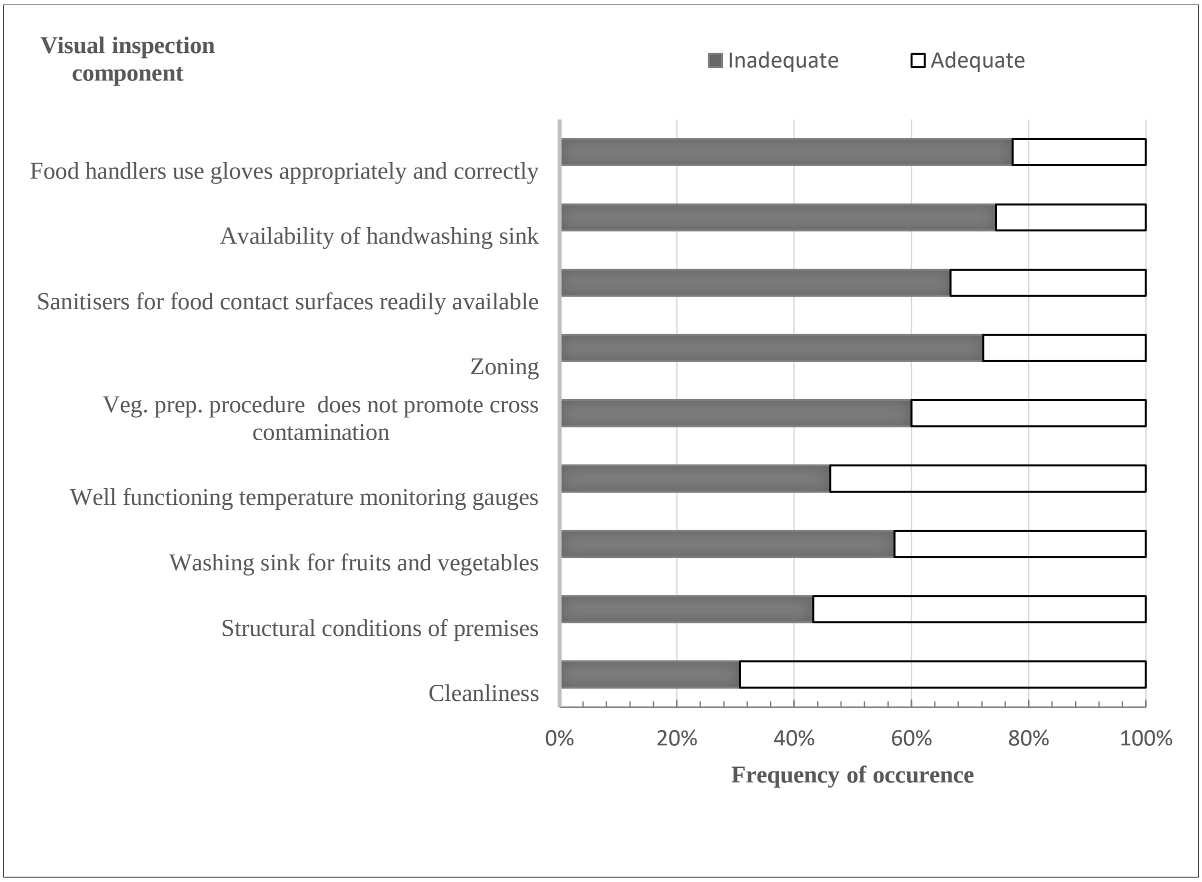


Figure 6.2. Distribution of food businesses' compliance with basic hygiene requirements and control measures

6.4.2. Handling practices and the process of salads vegetables preparation

Fresh vegetables were received during the mornings (7-9 a.m.) in plastic crates transported on open trucks or in vans. The great majority (95%) reported that they received fresh produce in uncooled vehicles (Table 6.1). In some cases, the person in charge or business owner purchased the daily needs from the central market or nearby groceries. More than two thirds of the respondents reported sourcing the fresh produce from the same supplier (68.4%), and washing the vegetables before cutting (77%). In general, preparation started early, particularly with bundles of parsley which were finely chopped for serving later in the day in traditional salads and appetizers.

Table 6.1. Frequency of self-reported handling practices of fresh vegetables in food service establishments

Process	Frequency of handling practices N (%)				
	Always	Often	Sometimes	Rarely	Never
Are fresh vegetables delivered from one supplier/source?	52(68)	17 (22)	5 (7)	1 (1)	1 (1)
Are fresh leafy vegetables or/and pre-cut vegetables delivered cooled?	2 (3)	0 (0)	2 (3)	0 (0)	72 (94)
Is the washing water used for fresh vegetables and fruits chlorinated?	13(17)	0 (0)	0 (0)	0 (0)	64(83)
Do you wash the vegetables before cutting?	51 (77)	1 (1)	1 (1)	0 (0)	13 (20)
If applicable: how often you record the temperature of the display salad bar?	12 (35)	0 (0)	0 (0)	0 (0)	22 (65)
The received fresh vegetables are kept in the cold storage room/fridge	67 (93)	0 (0)	1 (1)	0 (0)	4 (6)
The washed and cut vegetables for salads and garnishes are held at room temperature before preparation/service	17 (26)	0 (0)	2 (3)	0 (0)	47 (71)

Parsley leaves were chopped before washing in 34% of FSEs, which is consistent with the typical preparation sequence at homes (Figure 6.3), aiming to keep the texture of the leaves longer, as they would become soggy if they are washed ahead of time. About a third of the food businesses did not sanitize fresh vegetables, and used only water to wash them.

However, a large proportion (84%) reported that the wash water was neither treated nor filtered. With long-standing shortages of potable water in Lebanon, restaurants, and homes, purchase water to overcome the shortage in water supply, often of uncertain quality and source, which is then stored in tanks.

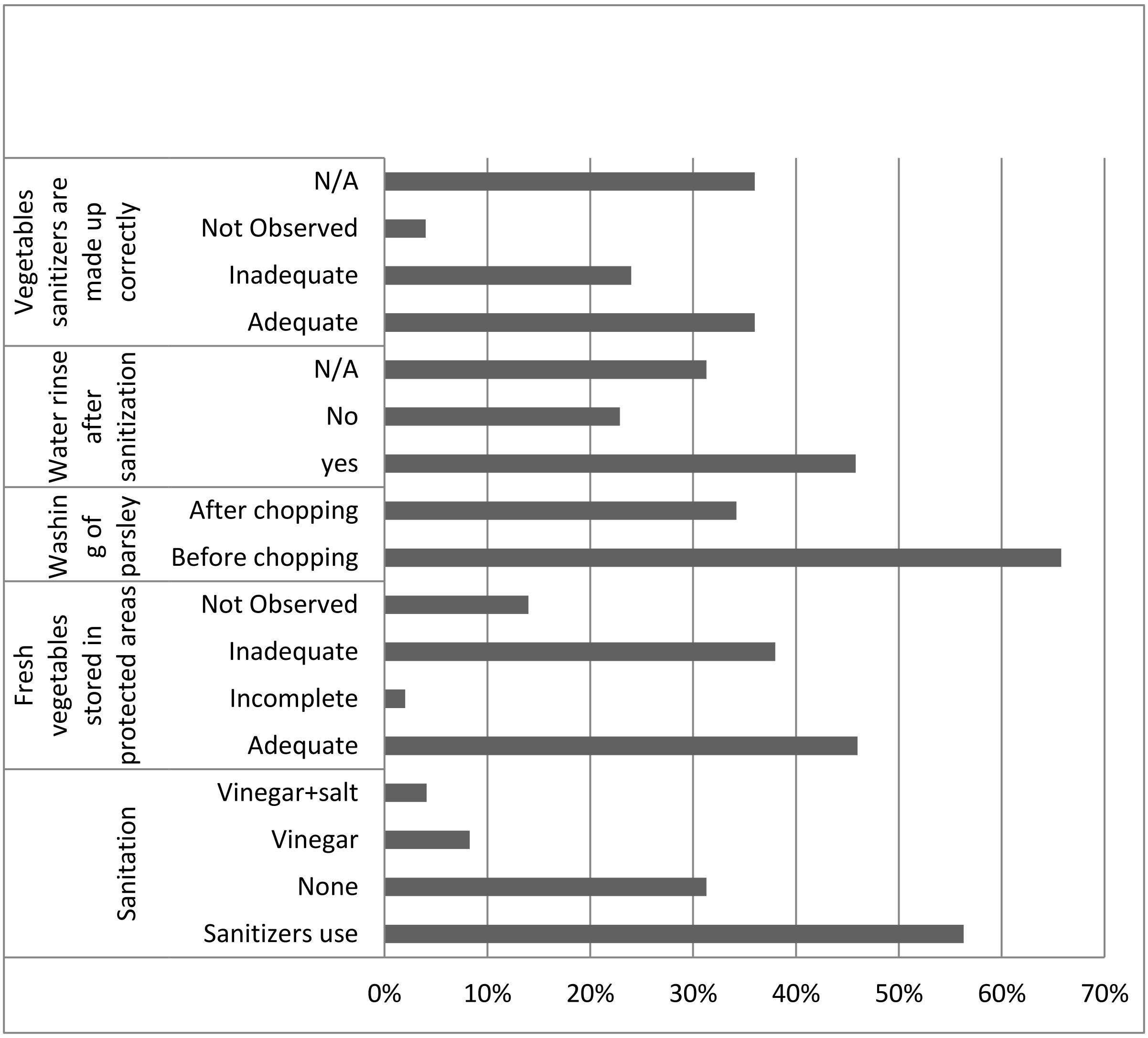


Figure 6.3. Distribution of food businesses' adequacy level in relation to washing and storing practices of fresh salads vegetables

Out of the 56% establishments using sanitizers, 21% used sodium dichloroisocyanurate (NaDCC) and nearly half (45%) applied a post-sanitization water rinse to remove the remaining taste or odor, respectively. It was noted during inspection discussions and observations that automated systems regulating the concentrations of chemical sanitizers in addition to water filters were in place, in some corporate-managed restaurants. In other places (24 %), incorrect dilutions of sanitiser were observed, typically as haphazard mixing of vinegar or NaDCC tablets in water. The majority reported that fresh produce was kept in cold storage, whereas this was actually only observed in 38% of the premises, with inadequate alternatives including stairways, kitchen floors of spaces in crowded production areas.

6.4.3. The microbiological quality of fresh salads vegetables

Results on microbiological analysis of fresh-cut salad vegetables are presented in (Table 6.2 and Table 6.3).

The mean APC levels ranged from 2.90 to 7.38 log CFU/g, with counts above 10^7CFU/g recorded for 17% of the samples. `The prevalence rate was substantially high for TCs (79.6%, 94/118). TCs were found between 1.72 - 6.40 log CFU/g, of which 38% were >4 log CFU/g. Whereas, *E. coli* was isolated from 31.3% (37/118), with bacterial loads ranging from less than 1.00 to 7.15 log CFU/g, and the incidence rate was 64.8% of the positive samples (24/37) for counts higher than 100 CFU/g.

More than two thirds (41.5%) of the samples were found to contain *S. aureus*. In addition, *Listeria* spp. were isolated from 70.6% of the samples. The overall incidence level was 53% for counts above 100 CFU/g, with an average of 3.24 log CFU/g. *L. monocytogenes* had a prevalence rate of 3.7 % mainly in arugula, parsley and lettuce, whereas *Salmonella* was detected in 0.9% (1/118), (lettuce).

Table 6.2. Microbial loads of different fresh salads vegetables

Produce	N	PCA†	Coliforms†
Lettuce	30	5.50 ± 1.55	3.89 ±2.19
Parsley	34	5.42 ± 1.32	4.48 ± 2.16
Cucumber	18	4.60 ± 2.01	3.52 ± 2.10
Radish	9	5.09 ± 2.20	1.72 ± 2.68
Mint	11	3.92 ± 2.74	3.93 ± 2.75
Coriander	1	7.38 ± 0.00	6.40 ± 0.00
Aragula	5	3.99 ± 2.44	3.30 ± 3.06
Tomato	3	2.90 ± 2.57	2.13 ± 2.20
Lettuce	4	5.35 ± 1.59	3.20 ±1.49
Iceberg	3	4.54 ± 0.77	1.46 ± 2.53

†Values are mean log CFU/g ± standard deviation.
The minimum detection limit was 10 CFU/g.

Table 6.3. Mean levels of *E. coli* and coagulase–positive *Staphylococcus* spp. on salads vegetables

Produce	N	***E. Coli*** log CFU/g ±SD (min-max)	***Staphylococcus* spp.** log CFU/g ±SD (min-max)
Lettuce	30	0.92± 1.80 (<1.00 -7.15)	2.89 ± 2.28 (<1.00 – 7.76)
Parsley	34	0.70 ± 1.50 (<1.00 - 5.40)	2.93 ± 187 (<1.00 – 6.16)
Cucumber	18	1.30 ± 1.43 (<1.00 - 3.40)	2.01 ± 1.99 (<1.00 – 5.45)
Radish	9	0.35 ± 0.88 (<1.00 -2.65)	2.84 ± 2.37 (<1.00 – 6.48)
Mint	11	1.36 ± 1.78 (<1.00 - 4.91)	2.69 ± 2.08 (<1.00 – 5.62)
Coriander	1	1.30 ± 0.91 (<1.00 - 1.30)	4.04
Aragula	5	0.92 ± 1.45 (<1.00 - 3.30)	2.76 ± 1.67 (<1.00 – 4.15)
Tomato	3	<1.00	2.00 ± 2.00 (<1.00 – 4.00)
lettuce	4	<1.00	4.47 ± 1.73 (2.30 – 6.00)
Iceberg	3	0.33± 0.58 (<1.00 – 1.00)	1.83 ± 1.58 (<1.00 – 2.78)

The minimum detection limit was 10 CFU/g.

Results on recovered microorganisms from contact surfaces (cutting boards and knives) are presented in Table 6.4. The microbial levels varied from below detection limits (10 CFU/swabbed area) to generally high levels. *E. coli* was isolated from 30.6% (15/49) of contact surfaces (knives and cutting boards); of those, the mean values were found between 2.70 - 7.02 log CFU/swabbed area, whereas the incidence rate in TCs was higher (53.0%, 26/49) with levels between 4.88 - 8.40 log CFU/swabbed area. There was no statistically significant correlation between the microbial counts recovered from contact surfaces and the ratings on the adequacy level of sanitation of work surfaces ($p > 0.05$).

Overall, the analysis of data showed no statistical significant differences and inconsistent trends in bacterial counts of different visual assessment rankings for each individual inspection component ($p > 0.05$).

Table 6.4. Bacterial counts recovered from two contact surfaces

Contact surface	n	Mean log CFU/swabbed area (min-max)			
		PCA	*Staphylococcus* spp.	*E. coli*	TC
Chopping board†	29	4.99 (<1.00-8.40)	4.42 (<1.00-8.40)	1.19 (<1.00-6.02)	2.62 (<1.00-8.40)
Knife*	20	5.62 (<1.00-8.40)	4.62 (<1.00-7.98)	1.13 (<1.00-5.95)	4.31 (<1.00-8.40)

†Cutting board swabbed area of 50 cm^2
*Knife (no defined area – ca.10-20 cm^2)

For instance, higher counts of TCs were observed on lettuce and parsley obtained from premises with inadequate sanitary conditions and unsafe handling practices, however, this was not the case with cucumbers (Table 6.5).

Also, the frequency in the distribution of bacterial levels on lettuce and parsley in relation to hygiene scores shows that high concentration levels were grouped at lower scores (Figure 6.4). Likewise, the mean levels of coagulase-positive *Staphylococcus* spp. were higher on all vegetables prepared on premises lacking handwashing sinks (Figure 6.5).

There was no correlation between total visual assessment scores and bacterial levels ($p > 0.05$). However, independent t-test still reveals a significant difference ($t=-2.198$, 81, $p = 0.03$), between inspection scores for premises with *Listeria* counts above 100 CFU/g (53.44± 18.39) and those where the organism was not detected (64.48 ±26.12).

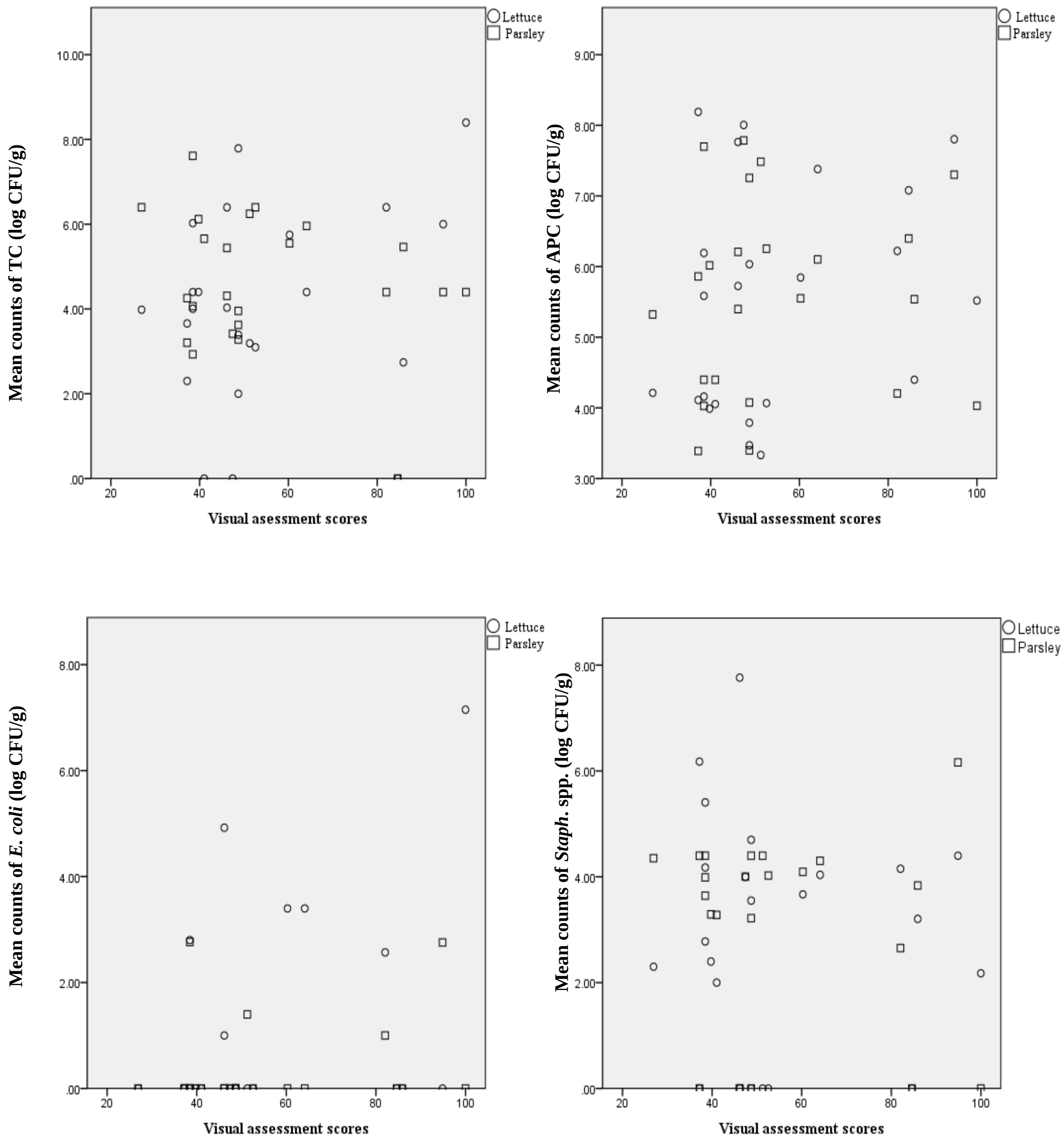


Figure 6.4. The distribution of microorganism levels on fresh vegetables in relation to the different values of visual assessment scores obtained on all inspected components

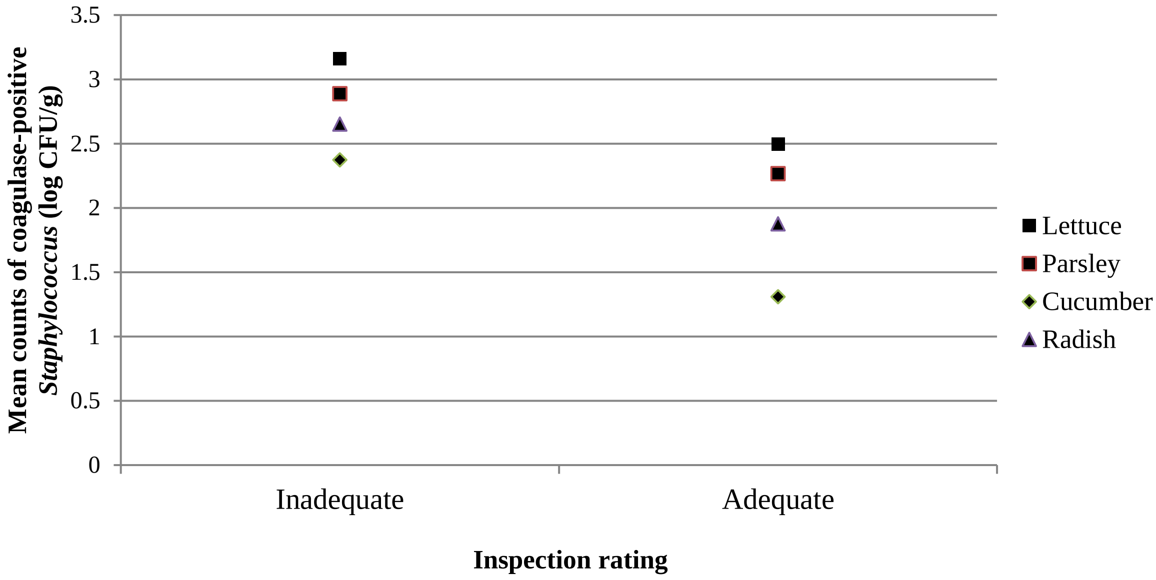


Figure 6.5. Distribution of mean levels of *Staphylococcus* spp. in relation to component "Availability of handwashing facilities"

When Eta correlation and non-parametric tests were further performed for this organism, no significant correlations of microbial results with all individual inspection component ($p > 0.05$) were shown, while correlation tests and cross tabulations somer'd test revealed a significantly low and moderate association of *Listeria* levels with the inspection components related to cross-contamination, handling practices, zoning and availability of handwashing sinks ($p < 0.05$) (Figure 6.6). This association level was consistent with linear regression indicating that *Listeria* spp. levels may be predicted by the visual assessment scores (F1,103) =11,614, p = 0.001, but the score accounted for only 10.5% (R^2) of the explained variability in *Listeria* levels in vegetables. Given the small value of R^2, the prediction model using the visual assessment scores is not accurate. However, and more interestingly, as each inspected component was considered individually, Eta^2 coefficients showed higher percentage in variations in *Listeria* spp. counts (30-34%) which were explained and attributed to cross contamination and cleaning operations components ($p < 0.05$).

Table 6.5. Distribution of the mean log CFU/g of bacterial loads on fresh produce according to adequacy level of control measures

			Prevention of cross-contamination	Sanitation		Protected, clean storage of fresh produce
Microorganism	**Rating†**	**N**	**Mean ± SD**	**Mean ± SD**	**N**	**Mean ± SD**
Coliforms						
Lettuce	Adequate	9	3.84 ± 3.09	3.67 ± 2.93	11	3.81 ± 2.59
	Inadequate	17	3.86 ± 1.68	4.20 ± 1.98	13	4.42 ± 1.68
Parsley	Adequate	10	3.80 ± 2.20	3.97 ± 2.23	14	3.95 ± 1.94
	Inadequate	20	4.68 ± 2.19	5.35 ± 2.39	13	4.46 ± 2.69
Cucumber	Adequate	6	4.15 ± 2.42	3.92 ± 2.48	7	3.84 ± 2.35
	Inadequate	9	3.79 ± 1.82	3.47 ± 1.99	7	3.61 ± 2.06
E. coli						
Lettuce	Adequate	9	1.46 ± 2.50	1.18 ± 2.17	11	1.19 ± 2.31
	Inadequate	17	085 ± 1.54	1.23 ± 1.77	13	0.85 ± 1.56
Parsley	Adequate	10	0.54 ± 0.97	0.79 ± 1.55	14	1.15 ± 2.05
	Inadequate	20	0.65 ± 1.48	0.81 ± 1.83	13	0.63 ± 1.15
Cucumber	Adequate	6	1.96 ± 1.47	1.79 ± 1.47	7	1.68 ± 1.53
	Inadequate	9	1.29 ± 1.43	0.91 ± 1.47	7	1.36 ± 1.53
PCA						
Lettuce	Adequate	9	6.14 ± 1.71	6.10 ± 1.54	11	5.41 ± 1.63
	Inadequate	17	5.21 ± 1.40	5.07 ± 1.32	13	5.41 ± 1.63
Parsley	Adequate	10	5.51 ± 1.51	5.48 ± 1.29	14	5.31 ± 1.28
	Inadequate	20	5.49 ± 1.21	5.30 ± 1.29	13	5.42 ± 1.55
Cucumber	Adequate	6	5.87 ± 1.22	4.36 ± 2.72	7	5.84 ± 1.11
	Inadequate	9	4.09 ± 1.82	4.84 ± 1.11	7	3.87 ± 1.96
Staphylococcus						
Lettuce	Adequate	9	2.83 ± 1.73	3.36 ± 2.13	11	3.20 ± 1.91
	Inadequate	17	2.67 ± 2.43	2.53 ± 2.55	13	2.84 ± 2.90
Parsley	Adequate	10	2.85 ± 2.17	3.16 ± 1.87	14	3.18 ± 1.89
	Inadequate	20	2.95 ± 1.78	2.26 ± 1.97	13	2.13 ± 2.08
Cucumber	Adequate	6	1.80 ± 2.02	1.56 ± 1.82	7	1.91 ± 1.87
	Inadequate	9	2.53 ± 2.12	3.24 ±.1.97	7	2.86 ± 2.12

† "Incomplete" ranking was omitted for easier presentation of data.

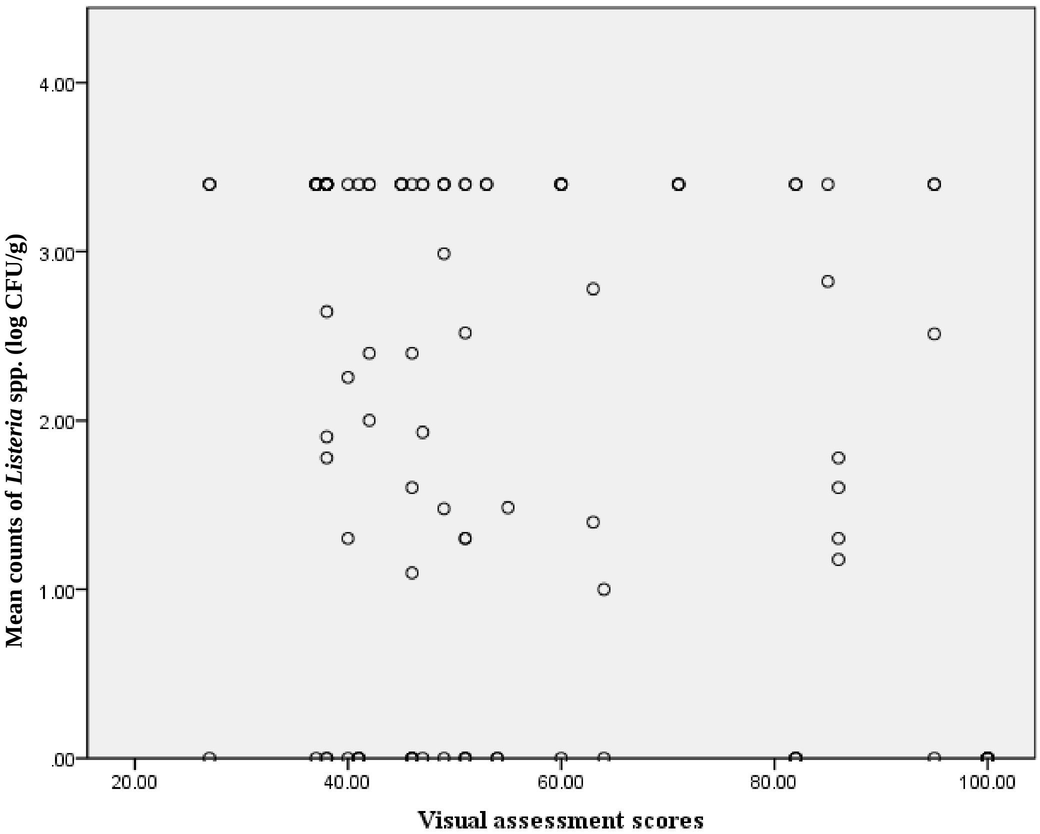


Figure 6.6. Distribution of *Listeria* spp. in relation to the visual assessment scores on all inspected components during salad vegetables preparation

6.5. Discussion

6.5.1. Food safety practices and microbial quality of fresh salads vegetables

A number of food safety practices concerns were identified in this study. The general lack of cleaning and sanitization procedures combined with a clear evidence of cross-contamination opportunities were generally reflected in the overall unsatisfactory quality of RTE vegetables. The majority of SMEs seemed to be unaware of the significance of applying control measures when handling vegetables and of the fundamental requirements for separate handwashing and vegetables washing sinks. Similarly, lack of sanitizers applications and standard operating procedures were noted in 16 food service operations by Strohbehn et al. (2011), and in their earlier study, when documentation and operating procedures were also not found in place, i.e., food workers did not record refrigerator and freezer temperatures (Sneed, Strohbehn, & Gilmore, 2004). In this study, APC were above the specified limits for RTEs, 7 log CFU/g, in 17% of the analysed samples. According to PHLS 2000 guidelines, aerobic mesophilic counts are not routinely performed for this category of food (e.g., RTE fresh vegetables) being part of the natural flora (PHLS, 2000). Hence, fresh salads vegetables are expected to have these microorganisms (Sagoo et al., 2001). APC were above the specified limits for RTEs, 7 log CFU/g, in 17% of the analysed samples. When APC count is $>10^6$ CFU/g, it may not necessarily relate to food safety hazards; in many of these cases, there is a predominant microorganism from an environmental source (PHLS, 2000), such as the processing stages involving handling, cutting, slicing and improper storage as well as display conditions (Abadias et al., 2012), whereas counts $<10^6$ CFU/g are usually associated with a mixed flora; Nguz et al. (2005) showed that chlorine treated fresh-cut organic mixed vegetables were still found to harbour high levels of TCs (5.9 log CFU/g) and it was proposed that high loads of coliforms in RTE vegetables at retails levels is directly influenced by intense use of untreated manure during pre-harvest, and extensive handling during postharvest (Aycicek et al., 2006), which brings us back to the TCs≥5 log CFU/g that were isolated from more than two third of the fresh vegetables (69%) coming from locations with alarming deficits at harvest and post-harvest washing, storage and distribution stages.

According to the EC legal food safety criteria and the UK Public Health Laboratory Service (PHLS) microbiological guidelines for RTE foods sampled at the point of sale, for category 5 fresh vegetables (PHLS, 2000; HPA, 2009), the study results on microbial contamination levels of more than half of the RTE salad vegetables were unsatisfactory due to *E. coli* and *Listeria* spp. counts that exceeded the criteria limits $>10^2$ CFU/g indicating poor hygienic practices and sanitary conditions (Gilbert et al., 2000).

Listeria spp. are rarely implicated in illnesses involving produce, however, they may indicate a significant failure of hygiene standards in the preparation and /or storage of fresh vegetables (Gilbert et al., 2000) which in turn are considered hazardous for *L. monocytogenes* contamination (Ponniah et al., 2010). *L. monocytogenes* and *Salmonella* spp. were traced back to samples obtained from restaurant that had no handwashing sinks, fresh vegetable washing sinks, or adequate preparation and storage areas or surfaces and the corresponding visual assessment score recorded 32 over 100 possible points.

The preparation of raw vegetables took place where meat and chicken in marinates were also prepared; and the sinks used for washing vegetables were found unfit. This is undoubtedly conferring further opportunities for cross-contamination. Several restaurant outbreaks in Oregon and Washington were linked with a variety of items from the salad bar and reported outcomes on investigations indicated that the trimming, macerating, and marinating the beef, which was obtained from the same source, took place in the same kitchens used for preparation of fruits and vegetables for the salad bar (Doyle et al., 2006). The limited space in SMEs in this study was a critical risk factor that could exacerbate the risk of cross-contamination, particularly when it leads to compromising the safety of fresh produce for the poor storage conditions and the lack of hand washing sinks. Underestimating the importance of proper storage for fresh produce was often reflected by the way fresh vegetables were place on floors, side streets at the restaurants entrance awaiting food workers. The misperception of risks among food handlers on that fresh produce should not be exposed to cold temperature was noted, when the storage of leafy greens at inadequate temperature in restaurants may lead to bacterial proliferation, and contamination (Khalil & Frank, 2010).

The lack of handwashing sinks explained the fact that proper handwashing before and after use of gloves was not commonly observed, although many other factors could interfere as well. High frequency of *S. aureus* indicates poor hygiene practices of food handlers, the latter being known to be carriers of this pathogen (Todd et al., 2008) and may contribute in direct contamination of RTE fresh vegetables and contact surfaces via the hands (Todd et al., 2008). It is important to mention again that *S. aureus* was highly prevalent on fresh produce collected from post-harvest stages and the wholesale market, the central source of produce to restaurants and groceries in Beirut.

6.5.2. Food contact surfaces

The PHLS recommended guidelines for cleaned contact surfaces specified levels of total viable microorganisms less than 80 CFU/cm^2 as satisfactory, 80-10^{3}CFU/cm^2 is borderline, and over 10^{3}CFU/cm^2 is unsatisfactory been associated with poor hygiene practices (Herbert et al., 1990). PCA counts $\geq$10^{3}CFU/cm^2 was recorded for 33/49 swabbed surface. The overall incidence rate of *E. coli* was 15/49 with counts $\geq$ 1 CFU/cm^2, whereas *E. coli* counts $\geq$10^{3}CFU/cm^2 were recorded for 10/49 of swabs. TCs and *Staphylococcus* spp. were found with counts $\geq$10^{3}CFU/cm^2 in 26/49 and 39/49 of swabs. In this regard, the high microbial population size on contact surfaces offered an additional assumption for the actual contamination observed on the washed salad items, particularly that sanitization and cleaning operations were lacking in a great majority of locations. It is worth noting that the efficiency of cotton swabs is limited as they generally remove <10% of the organisms present on the surface (Williams, 1967), hence the contamination levels are expected to be higher than the reported values.

Sneed et al. (2004) indicated that inadequate sanitation and recontamination problems were actually related to high aerobic plate counts recovered from cutting boards. Non-sanitized and scratched cutting surfaces, combined in some cases with misuse of sanitizers dilution, are an appropriate environment for harbouring pathogens that have the propensity to form biofilm on surfaces (Pui et al., 2011) and resist washing processes (Ravishankar et al., 2010). As critical as fresh-cut salads processing can be, FDA defined cut leafy greens as a potentially hazardous food (PHF) requiring time and temperature control for safety, thus developed the Guide to Minimize Microbial Food Safety Hazards of Leafy Greens (FDA,

1998) which also recommends training programs targeting leafy greens for all potential handlers of leafy greens. In this regard, it is relevant to note again on that less than half (42.5 %) of food handlers working on sampling locations were trained, yet demonstrated limited knowledge on temperature control and did not necessarily received guidance on handling fresh produce, whereas, the influence of training on safe handling of leafy greens and keeping records for traceability in accordance with the FDA guidelines have proved to lead to satisfactory trends in handling leafy greens (Coleman et al., 2013).

As RTE fresh vegetables were obtained after washing, the existing microbiological characteristics raise further doubts as to the implication of water quality. It is substantiated that washing with water of unsatisfactory microbial quality can serve as a vehicle for dispersion of microorganisms (Holvoet et al., 2013) and was the primary cause for the homogenous spread of *S.* Enteritidis to fresh-cut vegetables during processing (Perez-Rodriguez et al., 2014). The quality of water used for washing or in post-sanitization rinsing process in SMEs should be addressed in future studies as a critical element to maintain fresh vegetables safety specially when more restaurants nowadays rely on purchasing water of unknown sources, usually coming in tankers collected from spring water but may or may not be chlorinated, to compensate for the long-lasting shortage in water supply in many cities of Lebanon.

6.5.3. Association of microbial counts to visual assessment scores and inspection components

The data revealed an inconsistent association between the bacterial counts and visual assessment scores of handling practices and hygiene conditions. As the possibility of association to each single inspection component was also studied, the microbiological quality of salad vegetables did not show any direct correlation with each individual inspected component. It was found that the cell counts were either corresponding or conflicting in trend across ranking on adequacy level and types of produce. The complexity of the interfering factors during sampling of RTE fresh vegetables from different operational conditions, e.g., environment and storage temperature, receiving and pre-receiving conditions of fresh vegetables, preparation stages of fresh-cut vegetables, sampling methods, challenges the possibility to detect a clear-cut trend and association. Add to this, large number of samples might be needed to investigate such a trend. The present findings are in accordance with a study by Powell and Attwell (1995) where a link between the total viable counts and *S.aureus* on turkey and ham and the compliance rate to different inspection components was not established. Findings of earlier studies did not as well confirm such an association with the microbiological quality of foods of meat origin (Wyatt & Guy, 1980; Tebbutt & Southwell, 1989). Kuri et al. (1996) reported that microbial indicators in meats, including pathogen prevalence, were not correlated to total hygiene scores of meat retailers, nor to temperature of samples, but they were related to type of retailer or origin of product.

Higher population size of hygiene indicators was observed on some samples prepared under inadequate hygiene conditions, although a statistically significant correlation with the inspection scores failed. According to the results of this study, it may be reasonable to consider that low visual assessment scores on the hygiene standards and handling practices probably indicate unsatisfactory microbial quality and likelihood for risks of salad vegetables contamination with *L. monocytogenes*, however, this association was only significant in relation to individual components related to cross-contamination and effective cleaning. This present work concurs with studies of Leong et al. (2014) and Dauphin et al. (2001) where the application of Pulsed Field Gel Electrophoresis (PFGE) typing method provided an advantage of examining the contamination patterns and the prevalence of *L.monocytogenes* in food processing facilities. It was confirmed that contamination of the final products of smoked salmon originated from the processing environments rather than the *L.monocytogenes* on raw salmon (Dauphin et al., 2001). Similarly, Leong et al. (2014) demonstrated the persistent strains of *Listeria* spp. in the processing facilities and provided evidence of bacterial transfer from the processing environment to food.

During inspection, the total visual assessment score can be affected by a number of possible combinations of ranking levels of the 26 variables; a low inspection score might not necessarily indicate low ratings of all the critical components that have direct impact on the microbiological quality of vegetables. This study emphasized that inspections should focus upon factors most likely to be responsible for high microbial levels associated with RTE vegetables and the use of microbiological analysis of surface to check good hygienic practices and preventive measures.

6.6. Conclusion

Links between the visual assessment scores on the overall food safety performance and the microbiological quality of RTE fresh vegetables are not simple to establish and were not clearly correlated. The total visual assessment scores per se would not directly indicate the microbiological safety of RTE vegetables in restaurants. However, variations in microbial counts and a significant correlation of high *Listeria* levels with the inadequate cleaning performances and cross-contamination preventive measures were recorded, which imply that shortfalls in those particular practices may possibly indicate pathogenic contamination of fresh vegetables.

This study found high microbial loads in RTE vegetables that could serve as an indicator for the need to promote awareness on the critical areas commonly identified in SMEs and as guidance for local authorities to target those that may mostly affect the safety of fresh vegetables. Therefore, applications of critical control points for the preparation of fresh salad vegetables and personnel training on the hazards associated with their preparation are fundamental to improve the food safety of fresh produce particularly when prepared in small working facilities in SMEs.

It also underscored the considerable requisite for improvement in sanitary, storage and good hygienic practices. An emphasis should be placed on vigilant cleaning and sanitation procedures to reduce or eliminate contamination and cross-contamination risks that may occur at pre-farm gate and throughout the supply chain stages described in chapter 3. For this, an evaluation of the efficacy of the common sanitation and washing methods of fresh vegetables and contact in the forthcoming chapters is imperative in order to strategize applicable solutions in SMEs for reducing the risk of bacterial hazards during the preparation of salads vegetables.

7. The influence of pre-wash chopping and storage conditions of parley on the efficacy of disinfection against *S*. Typhimurium

7.1. Introduction

Fresh leafy greens continue to pose health risks due to its exposure to microbiological contamination though usage of untreated irrigation water (Pachepsky et al., 2011), inappropriate organic fertilizers and untreated manure, presence of wildlife or malpractices that can take place during harvesting, handling, transportation, processing and packaging (Olaimat & Holley, 2012). Hence, it is widely recognized that fresh produce is among foods that necessitate safe handling practices to prevent foodborne disease (McCabe-Sellers & Beattie, 2004). However, these are not easily achieved and despite the numerous studies and efforts to develop mitigation strategies, several outbreaks of human infections linked to consumption of fresh vegetables persist and have been increasingly documented worldwide (Buck et al., 2003; Sodha et al., 2011), but not in the Arab region, including Lebanon. These have been linked to norovirus, *E. coli* O157:H7, *Campylobacter, L. monocytogenes*, and *Salmonella* with the last being the most frequently encountered in outbreaks, especially linked to leafy greens (Patel & Sharma, 2010; Behravesh et al., 2011; IFSAC, 2015). *Salmonella* spp. are usually transmitted to humans by eating food contaminated with animal faeces (e.g., birds, domestic and wild animals grazing on crop fields); Some habitats, such as ponds and drainage ditches are also potential avenues for fresh produce contamination, besides the unhygienic hand contacts during the post-harvest practices though the food chain (Beuchat & Ryu, 1997; Buck et al., 2003).

Besides *E. coli* O157, *Campylobacter* spp., and *L. monocytogenes, Salmonella* is considered as the one of those most common severe pathogens associated with outbreaks linked to fresh produce (Behravesh et al., 2011; IFSAC, 2015), in addition to leafy greens being recognized as the leading source of food poisoning illnesses (Patel & Sharma, 2010).

Within the environment, *Salmonella* spp. are usually transmitted to humans by eating food contaminated with animal faeces (e.g., birds, domestic and wild animals grazing on crop fields), as well as water from ponds and drainage ditches in addition to unhygienic hand contacts during harvesting and post-harvesting practices though the food chain (Beuchat & Ryu, 1997; Buck et al., 2003).

Inadequate post-harvest cleaning procedures allow the bacteria remaining on surfaces of contaminated fresh vegetables to initiate growth when subjected to optimum conditions during handling and storage (Koseki & Isobe, 2005). There is a body of evidence that pathogenic microorganisms attached on the surfaces of vegetables particularly on cut surfaces are able to colonize in biofilms (Fett, 2000; Beuchat, 2002; Ells & Truelstrup Hansen, 2006; Tang et al., 2012) which could limit and interfere with the disinfecting efficacy of various sanitizers (Koseki et al, 2001b; Ölmez & Temur, 2010). Consequently, the survival of attached pathogens on fresh produce not subjected to subsequent heat treatment pose health risks to consumers.

Further down the produce chain, mishandling in food service operations has been linked to several reported food poisoning outbreaks involving fresh vegetables (CDC, 1999, 2007; De Jong et al., 2007; MacDonald et al., 2011). Investigations of outbreaks of foodborne disease in England and Wales (1992-2006) that were associated with ready-to-eat salads indicated that the majority of the outbreaks occurred in the food service and catering sectors and were linked to infected food handlers, cross contamination and poor storage (Little & Gillespie, 2008). The most common pathogen involved was *Salmonella* followed by norovirus (Todd & Greig, 2015). Figures for the developing countries such as those in the Middle East are relatively scarce on foodborne illnesses associated with the consumption of raw vegetables, although leafy vegetables such as parsley are often consumed raw in various traditional salad meals, or mezze garnishes. There are several risk factors that may contribute to microbial contamination of leafy greens from farms to wholesale and retail markets (as described in chapter 3 (Figures 3.2-3.4). One challenge is to find effective washing and sanitization procedures for fresh vegetables, as critical steps to ensure appropriate safety without adversely affecting the sensory, and nutritional characteristics of fruits and vegetables (Martínez-Sánchez et al., 2006). To this end, the use of sanitizing agents such as chlorine-based compounds, ozone, peroxyacetic acid, electrolyzed water, and organic acids in various postharvest operations is widespread (Kilonzo-Nthenge et al., 2006; Vandekinderen et al., 2009; Rahman et al., 2010; Ramos et al., 2014). However, chemical compounds based sanitizers such as the inorganic chlorine compounds have been reported to produce hazardous by-products (FDA, 1998a; Kim et al., 2012) and alteration of the food quality at doses permissible to eliminate pathogens (Beuchat & Ryu, 1997). Hence, Sodium dichloroisocyanurate (NaDCC) known also as Troclosene Sodium, has been advocated as an alternative to chlorine to treat water, with the advantage of leaving no odour or taste and prolonged effectiveness (Clasen & Edmondson, 2006).

NaDCC is a di-chlorinated isocyanuric acid derivative (FAO/WHO, 2008) that upon dissolving in water releases a variety of chlorinated and non-chlorinated isocyanurates and free available chlorine in the form of hypochlorous acid, recognized for its oxidation property and as a microbicidal agent (Clasen & Edmondson, 2006). Furthermore, it has been reported to be effective to sanitize fresh vegetables against *Salmonella* spp. (Nascimento et al., 2003). As efforts are concerted towards seeking new interventions and bio sanitizers, organic acids such as acetic and citric acids have been tested for removal of pathogens from fresh fruits and vegetables (Karapinar & Gonul, 1992; Wu et al., 2000; Rhee et al., 2003). These have been shown to be effective, convenient and economic to reduce microbial populations at the food service and household levels, with the additional advantage of a cleaner image. These sanitizers have to be effective enough to eliminate really low levels as few as 10 to 100 cells of *Salmonella* on parsley leaves which still constitute a potential health risk (Kisluk et al., 2012) and improper storage of cut produce can allow rapid growth of bacteria, as reported in an outbreak of salmonellosis in Germany that was traced to paprika with an estimated infective dose as low as 4 to 45 *salmonella* and stated by Kisluk et al. (2012).

Unfortunately, in many SMEs in the Middle East, these sanitizing agents are rarely used. In chapters 5-6, washing fresh vegetables with tap water was the most common method, followed with the use of a locally available commercial sanitizers, and vinegar. Parsley was often chopped before washing and in some cases kept on hold in warm ambient temperatures of 30-33ºC, in suitable conditions for pathogenic bacterial growth.

There has been no attempt so far, at least in the MENA to address the efficacy and safety of washing methods typically applied in SMEs on intact and cut parsley leaves *in situ* conditions. SMEs that serve raw parsley in ready-to-eat salads or sandwiches are popular, not only in Lebanon, but also for Syrian and Turkish food outlets. The aim of this study was to examine the effect of the pre-wash chopped parsley in different time-temperature conditions on the decontamination effect of simple and practical washing methods, with the view of supporting recommendations for safe handling practices of fresh leafy greens in SMEs.

7.2. Materials and methods

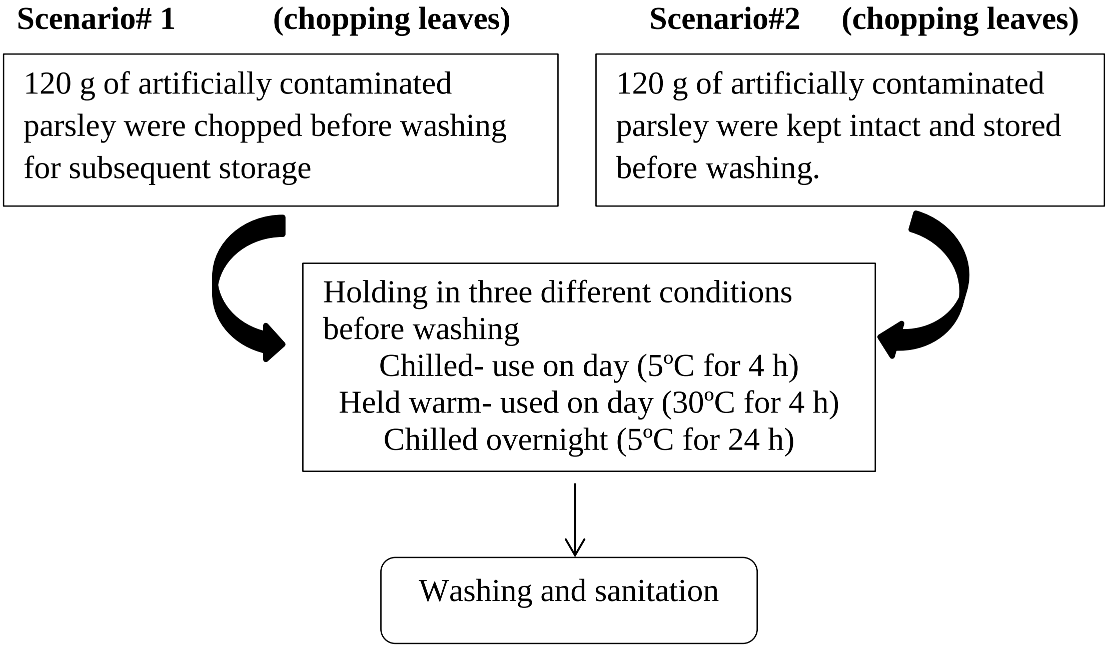
7.2.1. Preparation of parsley

Bundles of fresh parsley (*Petroselinum crispum*. var. *neapolitanum)* were purchased from a local retailer and used on the same day. Bruised and yellow leaves were discarded and the remaining intact green leaves were washed with running tap water to remove soils and dirt (Sengun & Karapinar, 2005; Ölmez & Temur, 2010) for approximately 1 min. Leaves were taken off the stems while keeping 2-3cm of the petioles, as prepared locally.

7.2.2. Rationale for the applied scenarios

The scenarios used in this study were based on observations derived from the observational assessment of food handlers' practices and handling of salad vegetables. It was noted that parsley is often chopped early in the mornings before washing, to preserve the leaves texture of the leaves by avoiding sogginess if chopped wet. It is held in uncontrolled environments, either in refrigerators or on shelves for variable periods until subsequent washing procedures, prior to serving at lunch or dinner services. In some cases, the chopped parsley was kept in a refrigerator until next day (when not served).

Washing was done by immersing parsley leaves in water for 15 min. followed by a rigorous manual agitation in the sink, then rinsing two or three times was observed in some small establishments, whereas others used NaDCC. A few outlets applied white vinegar in water for 15 min., however in unspecified and variable amounts. The experimental design resembled the same washing methods while maintaining the exposure time constant (15 min) for all solutions to observe of the effects of either chopping or not before washing, three different holding time-temperatures, and 5 different washing solutions on *S*. Typhimurium decontamination. The scenarios were as follows:



7.2.3. Preparation and application of washing solutions

Commercial white vinegar containing 5% acetic acid was purchased from a local supermarket and diluted with sterile water to prepare a solution of 4% acetic acid (pH 2.9). This concentration has been previously reported by Sengun & Karpinar (2005) and Ramos et al. (2014). A solution of 1000 ppm available chlorine (Chlor-Clean®, pH 5.94) and 0.25g/l NaDCC (Presept®, pH 6.14) were prepared. The 1000 ppm chlorine solution was included for reference (a concentration greater than 200 ppm of total chlorine is sufficient to achieve the desired sanitizing effect (FDA, 1998a). Deionized water (Milli-Q plus) was used for rinsing twice, with manual agitation (2-3 s in 3 successions). The pH of all treatment solutions was measured before and after 15 min. exposure.

The inoculated parsley (20 g) was immersed into 200 ml of each washing solution in sterile bags to cover all the leaves for 15 min at about 22ºC. After decanting (Lang et al., 2004), sterile bags were held upright in biosafety cabinet for 2-3 min., with additional light shaking to remove remaining drops of solutions on the leaves. All experiments were replicated at least 3 times and carried out in duplicate

7.2.4. Preparation of *S*. Typhimurium culture and cell suspension

Freeze-dried *S* Typhimurium LT2 was obtained from the School of Biological Sciences (Plymouth University). Cultures were grown from a stock kept at -80ºC, in brain heart infusion (BHI) broth overnight at 37ºC, streaked on blood agar plates and incubated overnight at 37ºC.

Then 1-2 colonies were cultured for 18 h at 37 °C in 10 ml tryptone soya broth (TSB) (Oxoid, Basingstoke, Hampshire, England) to provide an initial inoculum of approximately 10^9cells/ml grown to stationary phase, as confirmed by plating on Rapid'*Salmonella* agar. Bacterial cells are generally more tolerant than are logarithmic growth phase cells to environmental stresses (Miller et al., 2009) and *Salmonella* cells showed 1000-fold more acid resistance than logarithmic phase cells exposed to pH 3 for 1h (Lee et al., 1994).

Simultaneously, 1-2 colonies of the *S.* Typhimurium stock cultures adapted gradually to nalidixic acid (50µg/ml) (Bio-rad laboratories Ltd, Hemel Hempstead, UK) through stepwise exposure, i.e.,10, 20, 30, 40 and 50 µg/ml (Parnell et al., 2005). Afterwards, they were cultured by transferring to 10 ml TSB supplemented with nalidixic acid (50µg/ml) (TSBN) followed by incubation for 18 h at 37ºC. From these, cell suspensions of 10^6CFU/ml. and 10^3CFU/ml were prepared by 1000-fold dilutions of 1 ml and of a serially diluted 1 ml (10^{-6}) into 0.1% peptone water (PW), respectively.

7.2.5. Inoculation of parsley

Washed parsley was left to drain on sterile paper towels in a biosafety cabinet for approximately 1 h prior to dipping in inoculum suspensions (120g in 1l), containing targeted levels of bacteria, for 60 min with occasional manual agitation (Lapidot et al., 2006). After draining, the samples were dried on sterile towels in the biosafety cabinet for 1h at ambient temperature (22±1ºC). The target inoculation level was higher than the typically expected cross contamination levels, to allow an effective observation of bacterial reductions and the elimination effect of each factor. Considering the unlikelihood of high concentration of *Salmonella*, we examined the factors effects on parsley with low inoculum levels in two selected variables for additional validation of the trend in log reduction.

Control samples of unwashed inoculated parsley were taken before and after the washing procedures.

7.2.6. Detection and enumeration of *S*. Typhimurium

To determine the initial presence of *Salmonella* on parsley, homogenates of 25g in 225ml BPW (Biorad, UK) were incubated at 37ºC for 24 h, then 500 µl was transferred to 9.5ml selective enrichment in Rappaport Vassalidis (Biorad, UK) and incubated at 45ºC for 24 h. Later, a loopful of enriched solution was streaked on Rapid'*Salmonella* agar (Biorad, UK) for detection purposes.

S. Typhimurium enumeration was performed before and after the washing procedures. A 10 g sample of parsley was aseptically suspended in 90 ml of TSB in a stomacher bag and homogenized for 2 min.at 230 rpm. To determine the levels of *Salmonella* on the washed parsley, homogenates were serially diluted and 100 µl aliquots was plated in duplicate, in addition to 1 ml aliquots pipetted over 4 plates of PCA supplemented with 50µg/ml nalidixic acid. The background flora still overgrew the test pathogen in a number of replicates, which has been also reported by Gündüz et al. (2010). In preliminary trials on non-selective media (unreadable plates), the population size difference was 0.5 log CFU/g compared to selective agar and 1 log CFU/g for chlorine-treated samples. It is worth noting that the difference on the recovery of *Salmonella* cells between selective and non-selective media was reported to be insignificant by Gündüz et al. (2010). Therefore, to determine the survival of *Salmonella* enumerations were performed on a selective agar (Karapinar & Sengun, 2007; Patel & Sharma, 2010) where typical pink colonies were counted after incubation at 37ºC for 24-48 h.

Initial trials with nalidixic acid supplemented selective media to increase the selectivity resulted in smaller *Salmonella* colonies and occasional loss; hence we omitted this step as this medium was already highly selective.

To determine the viable uncultured cells on samples with inoculum of low levels (10^3), non-selective pre-enrichment, followed by selective enrichment according to ISO 16140 N° BRD 07/11-12/05 was performed.

Mean values of bacterial counts (CFU/g) from duplicate plate samples were log10 converted.

7.2.7. Scanning Electron Microscopy (SEM) of parsley leaves

The SEM imaging was performed to examine attachment of the test pathogen on the surface of parsley leaf to determine sites that featured preferential attachment and tor understand potential reasons for washing efficiency. The procedure of sample preparation for SEM examination was based on the protocol of Pathan et al. (2008) and on that described by Ells and Truelstrup Hansen (2006) and Ölmez and Temur (2010). Parsley leaves were removed from the bacterial suspensions after 24 h at 5ºC. Some leaves were treated for 15 min. by immersion in vinegar (4%) and in NaDCC (0.25g/l). Afterwards, leaves were rinsed twice in 0.1% peptone water(PW) and portions were immediately cut with sterile scalpel and sterilized cork-borer to the size of the stub diameter, and fixed for 2 h at 4 °C in 0.1 M cacodylate buffer, pH 7.2, containing 2.5% glutaraldehyde. Samples were rinsed three times with 0.1M Cacodylate, and then dehydrated by ethanol gradient series of 10, 30, 50, 70, 90, and 100%. The exposure in each step was 15-20 min with the final concentration being repeated three times for 30 min. Samples were then critical point dried with carbon dioxide (EMS Qourom 850) and mounted on specimen stub for coating with gold. Different locations of the samples were viewed using scanning electron microscope (Tescan Mira, Czech Republic).

7.3. Statistical analysis

A two-way ANOVA was used to assess the interaction of pre-wash parsley processing (chopping) and types of the washing methods on the reduction of attached *S*. Typhimurium using the IBM SPSS version 22. The level of significance for all tests was 0.05. When no interaction effect existed, simple main effects of each factor for the chopping process, for the different variables categories were examined by one-way ANOVA. The inspection Q-Q plots, tests for normality, examining standardized skewness and the Shapiro-Wilk tests were performed to check assumptions. As the analysis of variance is robust to violations of the homogeneity of variances, provided that the ratio of the largest group is not more than 3 times the smallest group, data were interpreted by Welch robust test and Games-Howell post hoc testing (Howell, 2007).

Independent t-tests were also performed for differences in mean values between both groups, chopped and unchopped, for each washing method treatment. The treatment effects on microbial loads were assessed by calculating the reduction of microbial content in relation to untreated samples, expressed as log-cycles, i.e. log (N/N_0) (Ramos et al., 2014), where N_0 is the sample initial microbial load and N is the microbial load after treatment.

7.4. Results

The results indicated no significant interaction (combination effect) between pre-wash processing of parley (chopping) and washing methods on reducing the number of *S.* Typhimurium counts in all tested conditions ($p > 0.05$), i.e., the pattern of change in *Salmonella* counts was fairly consistent across each type of washing solution on chopped and unchopped leaves. On the other hand, F-test results indicated that the main effects of the pre-wash chopping and types of washing methods were significant ($p < 0.05$). One-way ANOVA analysis showed that all types of washing solutions resulted in a significant reduction in mean values of *S.* Typhimurium on pre-wash unchopped parsley held at 5ºC for 4 h compared to control group ($p < 0.05$), which was also observed on unchopped parsley at 30ºC for 4 h and at 5ºC for 24 h ($p < 0.001$) (Table 7.1). On the contrary, both vinegar and water did not result in a statistically significant reduction in contamination levels on pre-wash chopped parsley compared to control group, with both inoculum levels and under all conditions (Tables 7.1 and 7.2). Overall, the difference among the mean values of all washing solutions was significant at low temperatures; Chlorine, followed by NaDCC, was the most effective in reducing contamination levels compared to vinegar and water (Table 7.2). This was also notable on unchopped and chopped leaves with low inoculum levels at 5ºC for 24 h ($p < 0.001$). However, NaDCC did not differ significantly from vinegar on unchopped leaves held at 30ºC for 4 h, and from chlorine on chopped parsley at 5ºC for 24h ($p > 0.05$).

Interestingly, the reduction in pathogen levels was not statistically different when comparing water and vinegar under all conditions ($p > 0.05$).

Table 7.1. Mean levels of *S.* Typhimurium (log CFU/g) on chopped and unchopped parsley leaves hold on different time-temperature conditions and washed applying different solutions

Pre-wash leaves preparation	Wash treatment		Pre-wash storage conditions (Temperature/Time)		
			5ºC/4 h	5ºC/24 h	30ºC/ 4 h
	Solutions	pH[a]	Mean±SD	Mean±SD	Mean±SD
Chopped	Control[b]		6.38 ±0.54	5.91 ±0.10	7.14 ± 0.44
	Water	6.30	5.44 ±0.36	5.64 ±0.27	6.55 ± 0.48
	Vinegar	3.65	6.02 ±0.55	5.32 ±0.47	6.60 ± 0.25
	NaDCC	6.09	3.99* ±0.79	3.62* ±0.12	5.21* ± 0.19
	Chlorine	6.11	2.51* ±0.28	2.64* ±0.45	4.19* ± 0.11
Un-chopped	Control[1]		6.20 ±0.53	5.84 ±0.09	6.98 ± 0.45
	Water	6.45	4.69* ±0.38	4.74* ±0.13	6.16* ±0.22
	Vinegar	3.49	5.12* ±0.51	4.66* ±0.12	6.11 ± 0.61
	NaDCC	6.10	3.08* ±0.45	3.18* ±0.27	5.09* ± 0.20
	Chlorine	6.00	1.28†* ±0.80	2.55* ±0.63	4.26* ± 0.21

[a] Mean pH of washing solutions decanted after the 15 min. values consistent for all settings and over time.
[b] Mean value of attached cells after holding under tested conditions and the initial inoculation with 10^6 CFU/g
* The mean value is significantly lower than the control group at $p < 0.05$ (significant difference from control) for each tested variable.
† For 2 out of 5 replicate experiments, no growth of *Salmonella* was noted after enrichment (no-detection limit < 0.7 log CFU/g).

Table 7.2. Log reduction (log N/N$_0$) of *S.* Typhimurium on pre-wash chopped and unchopped parsley inoculated with low inoculum levels† under selected temperature/time conditions

	5ºC/24 h	5ºC/ 24 h
Washing method	Unchopped log (N/N$_0$)	Chopped log (N/N$_0$)
Water	-0.98	-0.48
Vinegar	-1.25	-1.14
NaDCC	-1.85^{b}	-1.71*
Chlorine	-2.27*^{c}	-2.62*^{d}

†Mean values for control for whole leaves parsley at 5°C/24h and for chopped leaves at5°C for 4 h were 4.08 and 3.85 log CFU/g, respectively
The star on mean values indicates significantly lower mean compared to control group ($p < 0.05$).
Minimum detection limit was set to 0.7 log CFU/g to avoid under or overestimation in statistical analysis
^{b,c} In 1 out of 4 replicated experiments showed undetectable levels (≤ 0.7 log CFU/g for low inoculum). Detection test was positive after enrichment.
^{d} 2 out of 4 replicates showed undetectable levels (≤ 0.7 log CFU/g for low inoculum). Detection test was positive after enrichment

7.4.1. The effect of the pre-wash chopping process

Unchopped parsley washed by soaking for 15 min. in water followed by manual agitation after holding at 5ºC for 4 h and 24 h had a statistically significant lower contamination level compared to chopped leaves with a mean difference of 0.76 log (95% CI, 0.17-1.34) and of 0.898 log (95%CI, 0.47-1.32), respectively.

Similarly, vinegar was more effective on unchopped than on chopped parsley held at 5ºC for 4 h with a mean difference of 0.898 log (CI95%, 0.12-1.67) as illustrated in Figure 7.1.A. On the contrary, it was found that application of vinegar did not result in a significantly different contamination level between both groups when parsley was held at 30ºC for 4 h and 5ºC for 24 h, ($p > 0.05$) (Figure 7.1.B-C).

The effect of NaDCC and chlorine also differed significantly in both groups at 5ºC for 4 h; NaDCC (approached significance, $p = 0.056$) with a mean difference 0.91 log (CI95 %, -0.02-1.84) and chlorine with a mean difference 1.22 log (CI 95%, 0.24-2.2). But when inoculated parsley was held for 20 h more at 5ºC, chlorine did not result in any further significant difference in reduction levels between both groups.

In general, when unwashed parsley leaves were kept at 30ºC for more than 2-3 h, the mean values in both groups did not differ significantly for all washing solutions. The maximum reduction was mainly achieved on unchopped parsley, particularly with NaDCC and chlorine. This trend was also observed on samples with low inoculum levels (Table 7.2). NaDCC was capable of reducing the initial inoculum levels to an undetectable level in one sample of unchopped leaves (Table 7.3). Furthermore, water and vinegar did not have a significant decontamination effect on chopped parsley compared to control.

7.4.2. The main effect of temperature/time conditions

There was a statistically significant interaction effect of temperature and chopping process before washing, ($p < 0.001$).

Pairwise comparisons showed that *S.* Typhimurium counts were significantly reduced ($p < 0.001$). after washing unchopped parsley held at 5ºC for 4 h and 24 h compared to 30ºC for 4 h, with a mean difference of -1.647 and -1.528 respectively.

Further analysis revealed that the mean values in chopped and unchopped parsley held at 5ºC for 4 h and for 24 h were significantly lower ($p < 0.05$) than those held at 30ºC for 4 h for all washing solutions groups indicating the pivotal role of temperature in altering the washing solutions efficiency relatively to other assessed individual factors (Table 7.3).

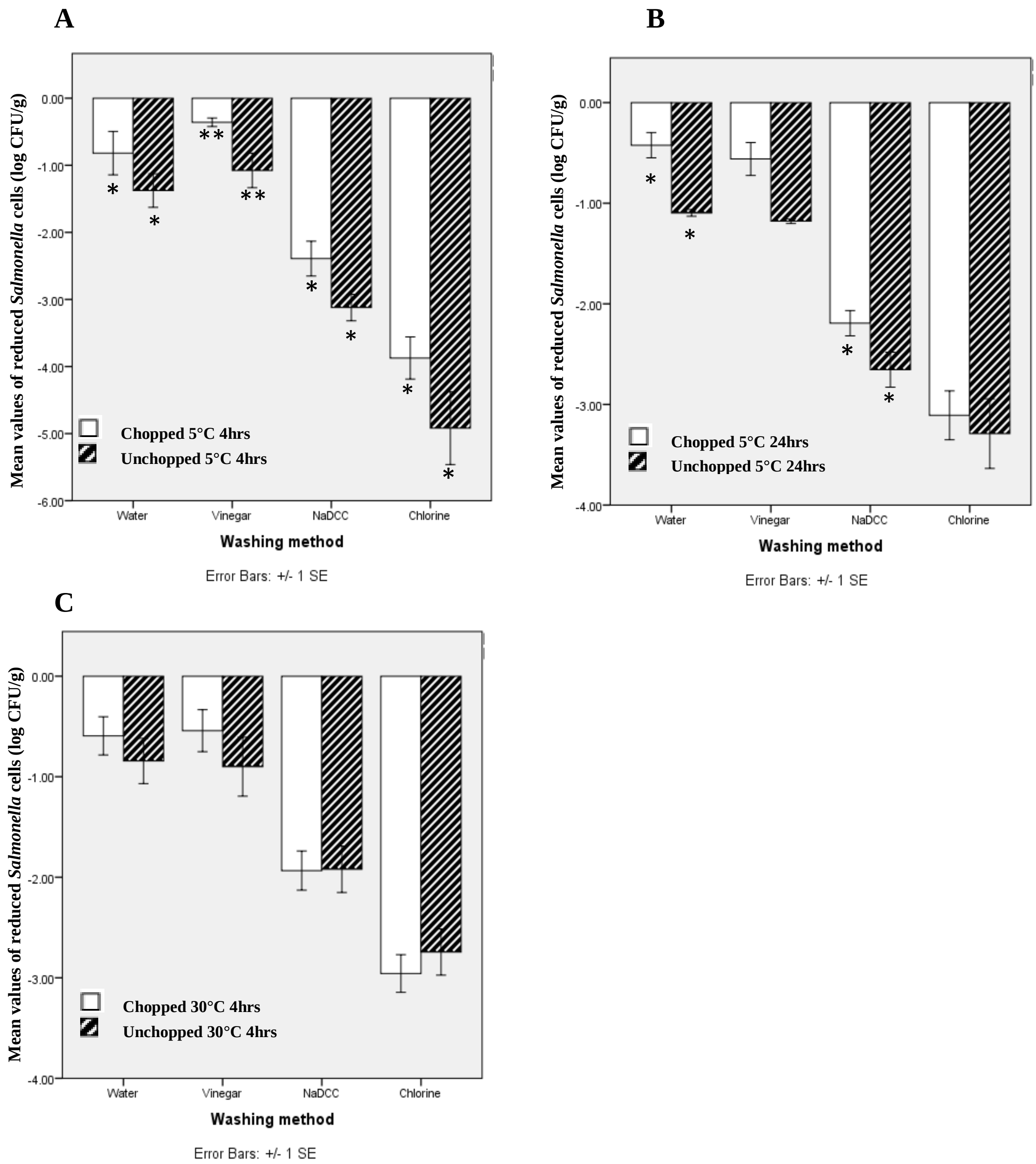


Figure 7.1.A, B, C. The differences in log reduction (log N/ N_0) of *S.* Typhimurium between chopped and unchopped leaves after treatment with washing solutions.

Bars noted with a star indicate a statistically significant difference between both groups (*) in each treatment category ($p < 0.05$).

** The difference between both groups approached significance ($p = 0.056$).

7.4.3. Scanning Electron Microscopy

The SEM imaging of presumed *S.* Typhimurium on parsley samples incubated for 24 h at 5ºC demonstrated what appeared to be clusters of cells agglomerated in the netting and crevices of small veins of the parsley leaf (Figure 7.2). There has been no clear indication of a preferential adhesion of colonies at the cut edges of the leaf as there was not any substantial and clear indication for a differential attachment around the scar.

There was apparently an initiation of formation of polysaccharide matrix and strands after 24 h incubation at 5ºC that held cells together and to the plant tissue (Figure 7.3). The observed clusters were apparently embedded within the folds and capable of evading most commonly used washing solutions (Figure 7.4), having being adhered to inaccessible sites on the leaf surface.

Table 7.3. The difference in log reduction (log N/N_0) of *S.* Typhimurium on parsley among the different temperature/time conditions of each group (pre-wash chopped and unchopped)

	Pre-wash storage conditions (Temperature/Time)					
	Chopped			Unchopped		
	5ºC/4h	5ºC/24 h	30ºC/4 h	5ºC/4 h	5ºC/24 h	30ºC/4 h
Washing solutions	log (N/N_0)	log (N/N_0)	log (N/N_0)	log (N/N_0)	log (N/N_0)	log (N/N_0)
Water	-0.93^{a}	-0.27	-0.59^{b}	-1.51^{a}	-1.098^{a}	-0.84^{b}
Vinegar	-0.36	-0.59^{a}	-0.54^{b}	-1.08^{a}	-1.18^{a}	-0.90^{b}
NaDCC	-2.39^{a}	-2.19^{a}	-1.93^{b}	-3.12^{a}	-2.65^{a}	-1.92^{b}
Chlorine*	-3.87^{a}	-3.11^{a}	-2.96^{b}	$-4.92^{a\ddagger}$	-3.29^{a}	-2.74^{b}

Different superscript letters in each row of each group (chopped and unchopped) indicate significant differences in mean values at $p < 0.05$

*Games-Howells post hoc test performed assuming non-variances. For remaining variables, Tuckey post-hoc test was run.

‡ 2 out of 5 replicated experiments showed no visual growth of *Salmonella* (undetectable levels ≤ 0.7 log CFU/g) after selective and non-selective enrichment. Mean value of log reduction would be equal to -3.7 if zero values were given in the event of undetected cells.

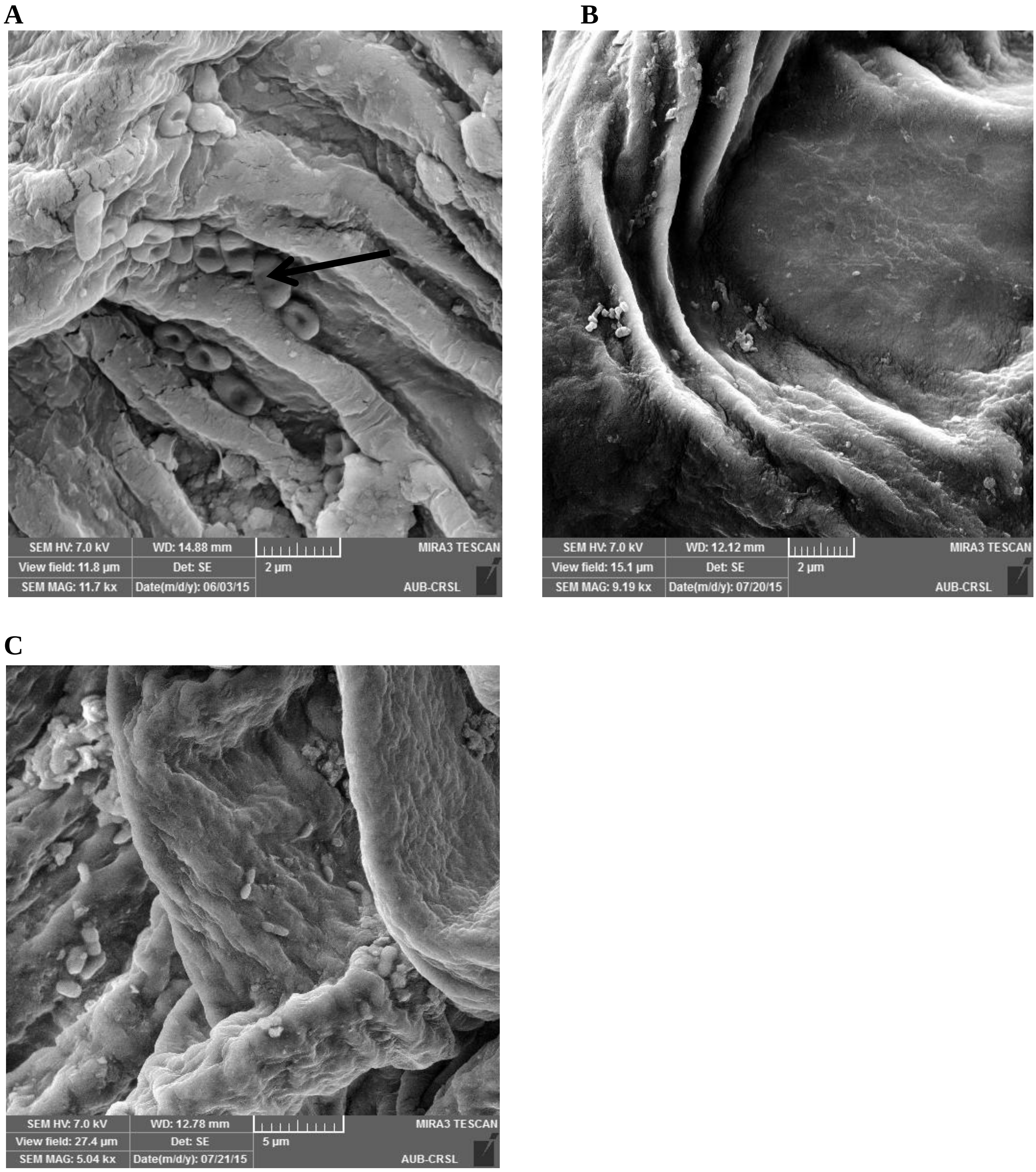


Figure 7.2. SEM micrographs of cells attachment in the inner folds of parsley leaf
S. Typhimurium agglomerated at the inner sides of small veins and crevices of the parsley leaf (A). This SEM micrograph taken from a view field of 15.1 µm showing clusters of presumed *S.* Typhimurium located mostly on the inner sides of the leaf veins (B) SEM image locating *S.* Typhimurium cells at the edge tip of leave (V shape) and shows that cells are preferably attached on folds of the small vein of a leaf cutting (C)

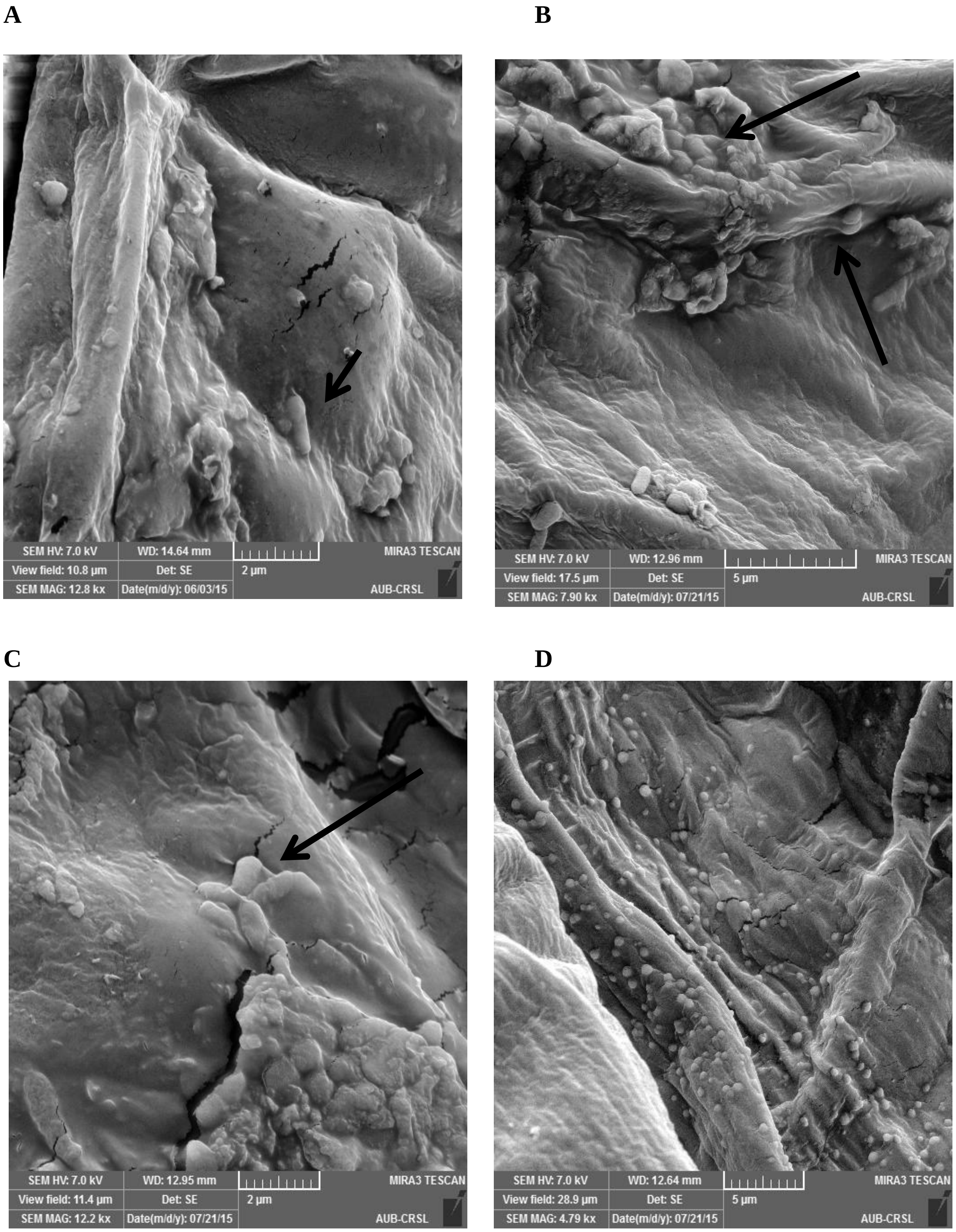


Figure 7.3. SEM micrographs of a biofilm initiation on the surface of a parsley leaf after 24 h storage at 5°C

They indicate the initiation of the formation of extracellular polysaccharide matrix in the netting of the inoculated parsley leaf. Arrows show strands of materials holding the cells to the parsley leaf surface. Planktonic cells were observed on crevices of the small vein of the leaf (A-B-C). The surface of a biofilm, a hydrated matrix of polysaccharide and protein formed by aggregates of bacteria (D).

7.5. Discussion

According to the present results, the current washing methods applied in the SMEs using water and vinegar were only capable of ≤ 90% reduction of the contamination level on intact and chopped leaves which is not considered sufficient to ensure microbiological safety given the very low infectious dose of *Salmonella* as well as the practice of uncontrolled dilution of vinegar typically used in restaurants.

Water wash achieved negligible log reduction with a range of 0.59-0.93 and 0.84-1.5 log for pre-wash chopped and unchopped leaves, respectively, which is in agreement with several authors. Sengun and Karapinar (2005) reported a 0.5–1 log reduction for wash with sterile water. Similarly, Neal et al. (2012) recorded only 0.7 log reduction in *Salmonella* with water wash of spinach, whereas a lower reduction was reported elsewhere (Tan et al., 2015). The higher numbers observed in unchopped samples might be due dislodging more cells by the rinsing in conjunction with successive rigorous agitation. The exertion of additional physical cleansing such as scrubbing in water was shown to increase reduction in log CFU/g compared to soaking (Parnell et al., 2005).

The studies on the decontamination effect of vinegar on produce, particularly on parsley, are very limited (Karapinar & Gonul, 1992; Wu et al., 2000; Sengun & Karapinar, 2004) and gave varying results. In this study, log reduction with vinegar achieved a maximum reduction of 0.54 and 1.08, for chopped and unchopped leaves, respectively. With similar concentration and exposure time, Sengun and Karapinar (2004) showed a maximum reduction of 1.87 log CFU/g and 2.45 log CFU/g with low inoculum levels of *S.* Typhimurium, in contrast to higher reduction levels on rocket leaves obtained in their other study. It is assumed that the lower values obtained in this study were attributable to attachment time of inoculum and to topography of parsley surface characterized by folds and niches that shield bacteria from treatment accessibility.

As *Salmonella* can survive and grow in a wide range of pH (4-9), besides that the pH value of vinegar was constant in all tested conditions, it is postulated that properties other than acidity of vinegar (hydrogen ion effect) underlie its effect on reducing the cell counts, such as the antimicrobial properties of phenolic compounds naturally existing in grape juice (Rhodes et al., 2006; Oliveira et al., 2013). Overall, this study has confirmed the equivalent efficacy of water and vinegar (4%) and the unlikelihood to reduce the numbers of bacteria by more than 1-2 log (Nastou et al., 2012). Although the US Environmental Protection Agency (EPA) Scientific Advisory panel proposed that at least a 2 log microbial reduction is considered as significant (São et al., 2015), the food safety laws require strict sanitation measures to achieve a reduction of 99.99683% (Fallik, 2004) which remains a challenge for SMEs in view of limited practical washing methods.

This study showed that NaDCC was the most effective method against *S.* Typhimurium with a log reduction range of 1.92-3.12. Its affordable price and convenience offer SME's with limited resources a practical alternative for fresh produce sanitation. There are few documented reports on the use of sodium dichloroisocyanurate in fresh produce (Nicholl & Prendergast, 1998; São et al., 2015; Tan et al., 2015), particularly in eliminating *Salmonella* on parsley. A log reduction of 99-99.99% was readily achieved in this study, which was consistent with a recent work on *S.* Typhimurium on turnip by Tan (2015). The effectiveness of NaDCC (200 mg/l) on other species was also demonstrated but in varying level indicating that the sanitization effect varies depending on the produce type, contamination and attachment levels and, bacterial species.

In general, all washing methods, with exception to chlorine, failed to eliminate *S.* Typhimurium, with high and low inoculum levels, with exception to few cases where NaDCC and chlorine reduced the pathogen to below the detection limit. It is thought that the inaccessibility of washing solutions to crevices and folds on parsley surface, hydrophobic pockets where bacteria hide and attach (Adams et al., 1989) in addition to the strength of attachment undoubtedly contributed to reducing the efficacy of sanitizing treatments as previously suggested by Ölmez and Temur (2010).

Interestingly, the effectiveness of washing solutions significantly dropped on samples subjected to pre-wash chopping and notably as storage temperature increased to 30ºC. The decrease in the initial inoculum levels was generally more significant on intact parsley leaves than on chopped samples with all washing methods. These results are in line with Patel who observed higher numbers of *Salmonella* attached preferentially to produce with a damaged surface, perhaps due to stronger binding properties on cut leaves. There are hypothetically a number of possible reasons for this. It is increasingly evident that *Salmonella* are capable of adherence to fresh produce surface (Ells & Truelstrup Hansen, 2006; Patel & Sharma, 2010). Additionally, the tissue damage and release of exudates by slicing, peeling or shedding of plant tissues produce abundance of nutrients to enteric bacteria enabling the cells growth on the produce (Sapers, 2002; Sela & Fallik, 2009). It is also substantiated that cutting plant surfaces resulted in larger surface area that support higher attachment levels (Ells & Truelstrup Hansen, 2006). It is however noteworthy to mention that other authors stated that *S.* Typhimurium did not differ in attachment strength to cut and intact lettuce at 4ºC for 8h (Takeuchi et al., 2000; Kroupitski et al., 2009). In this context, several citations shed the light on the complex attachment mechanism influenced by produce types, exposure time to contamination and strains (Reina et al., 2002; Patel & Sharma, 2010) and further influenced by the physiological state of the strains (Rees et al.,1995). Previous exposure to biocides which resulted in changes on proteins, bacterial cell adhesion properties, and their interactions with EPS that could in turn induce biofilm-mediated resistance has also been reported (Condell et al. 2012).

Images obtained by SEM indicated the adhesion of presumed *S.* Typhimurium and clusters of cells within inner folding of the veins on the surface of inoculated parsley held for 24 h at 5ºC (Figure 7.2A) which is likely due to the adhesion of bacteria. There was not a preferential attachment or clusters of cells anchored at the cut edges as hypothesized. The observation corroborates with Takeuchi et al. (2000) who demonstrated by means of a confocal scanning laser microscopy (CSLM) that different species of microorganisms attach differently to lettuce structures and *P. fluorencens* attached preferentially to intact surface than to cut edges. Apparently, there was a constant trend, although not significant, of a diminishing decontamination effects on parsley held at 5ºC for 24 h than for 4 h. It is well established that longer attachment time allowed more cells to attach, which is thought to be due the development of cell aggregates and biofilm formation that confer *Salmonella* its resistance to disinfectants and conventional household methods of washing (Takeuchi et al., 2000; Burnett & Beuchat, 2001; Koseki & Itoh, 2001; Lapidot et al., 2006).

The higher reductions observed on samples at 5ºC 24h treated with vinegar were negligible and might be due to roughness and folds of the parsley surface that led to minor variations among replicated experiments. It is conceivable that the declining pattern as validated with high chlorine concentration resulted from formation of extracellular polymers and increase in attachment with time (Reina et al., 2002; Ölmez & Temur, 2010). The SEM micrographs Figure 7.3 show cell clusters of presumptive *Salmonella* possibly on the initial formation stages of exopolysaccharide matrix (biofilm) and strands connecting cells together and to the plant tissue. Embedded cells inside the matrix on parsley surface were most probably able to escape effective contact with washing solutions, hence complete elimination by sanitizing agents was not observed in this study (Figure 7.4).

The decontamination effect of all solutions was the least effective at higher temperature (30ºC) perhaps because of a lower permeability of treatment in view of increased cells attachment. It is generally agreed that low storage temperature 4 °C suppresses the microbial growth (Dinu & Bach, 2011; Tan et al., 2015). Nevertheless, review of literature reflected the complexity of the attachment process as affected by temperature conditions and the disparities in several suggested underlying mechanisms. Ells and Truelstrup Hansen (2006) indicated that at 37ºC, cells exhibited significantly lower attachment strengths during the first 4 h given the lack of production of flagella at this temperature. Earlier, Herald and Zottola (1988) reported on the effect of flagella on attachment. Findings showed an increase production of flagella with the decrease in temperature hence the decreased numbers of bacterial attachment at low temperature. Whereas, Reina (2002) proposed that binding strength increases with contact time, but a temperature dependent response was mainly noted in the early stages of exposing the produce surface to inoculum. Recently, Patel and Sharma (2010) pointed out that low temperatures and short periods of contact with the produce surface will reduce the potential for bacterial adhesion; at the same time, the increase level of attachment of *Salmonella* at higher temperature was proven (McAuley et al., 2015). It is believed that this effect is due to a decrease in the bacteria surface polymer at lower temperatures as well as to reduced surface area (Garrett et al., 2008). On the other hand, Stepanović et al. (2003) stated that optimum temperature results in rapid bacterial growth and biofilm formation of bacteria in association with an increase in nutrients due to increase in the bacterial enzymatic reactions which control the development of many physiological and biochemical properties of bacteria (Garrett et al., 2008). It is perhaps not easy to ascertain the precise mechanism of the study results as several factors could have been possibly involved, nevertheless, the alteration in efficiency of washing methods by temperature and chopping practice was verified.

A B

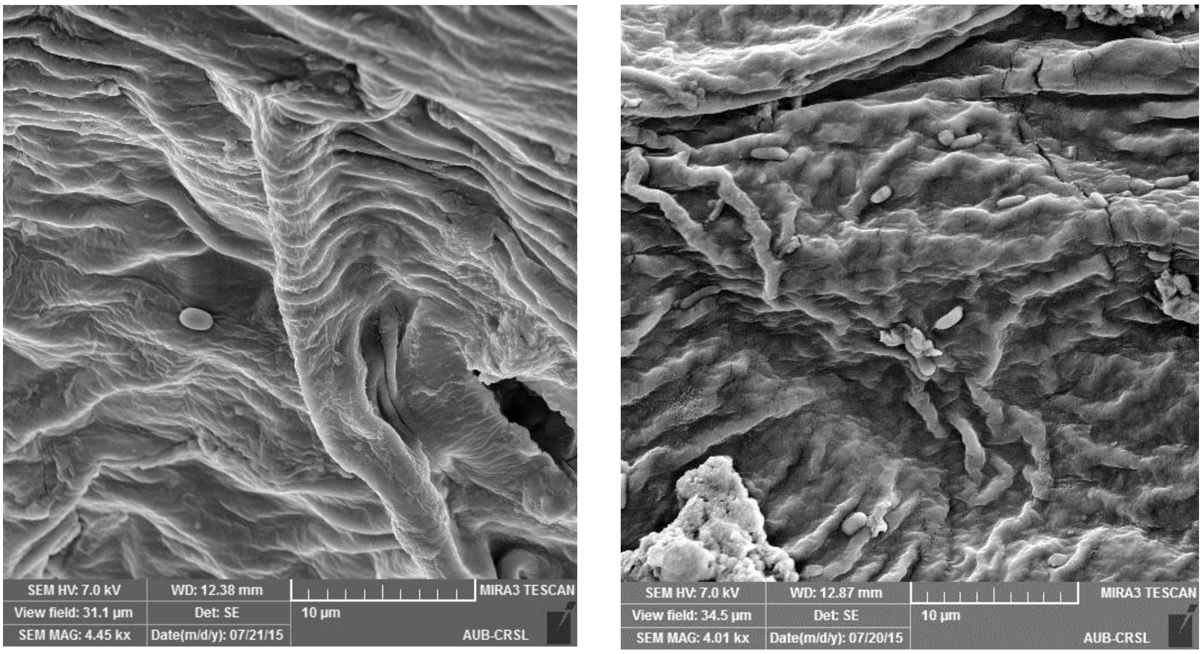


Figure 7.4. SEM imaging of inoculated parsley leaf after immersion for 15 min.in NaDCC (A) and in vinegar (4%, v/v, acetic acid) (B).

7.6. Conclusion

The findings of this study highlighted the importance for temperature control over time during handling of parsley for the optimal elimination of pathogenic microorganisms. It demonstrated that chopping parsley leaves before washing and sanitization, and storing them at inappropriate temperature would reduce the effectiveness of washing procedures typically applied in the SME's by 0.5-1.9 log compared to cold storage temperature. Since *S.* Typhimurium has the ability to persist in soils contaminated by manure or irrigation water and contaminate parsley (Kisluk et al., 2012) and is not eliminated by the inappropriate post-harvest washing and employee mishandling, it is critical that the most effective sanitizers are used during parsley in the food service operations. Chlorine compounds are the most effective and economic to use but are avoided by many facilities because of their undesirable sensory characteristics. The results showed that NaDCC is an acceptable substitute to chlorine and other tested solutions that should be used to intact leaves stored under controlled temperature and storage conditions. Its use could be as well advocated by local authorities as an alternative sanitizer for reducing risks of foodborne illnesses associated with consuming raw parsley and other leafy greens in FSEs. This is the first study to evaluate the effectiveness of common washing methods against *S.* Typhimurium in scenarios that represent SMEs practices in the Middle East/MENA region for processing raw parsley.

8. The transfer rate of *salmonella* Typhimurium from contaminated parsley to other consecutively chopped batches via cutting boards under different food handling scenarios

8.1. Introduction

Many strains of *Salmonella* pose a global health threat for foodborne disease including *S.* Typhimurium (CDC., 2006; EFSA, 2010). At the same time, the health concerns are becoming significant given the increasing prevalence of multidrug-resistant *S*. Typhimurium infections in many parts of the world (Kumar et al., 2008; Dutta et al., 2014). A quarter of a century ago, Madden (1992) stated that fresh produce should be defined as potentially hazardous food. It is actually becoming more evident that *Salmonella*-associated outbreaks are not limited to contaminated foods of animal origin; they are periodically linked to consumption of fresh produce (Jackson et al., 2013), including parsley and lettuce (Lapidot et al., 2006; Berger et al., 2010; Pui et al., 2011) and *S.* Enteritidis and *S*. Typhimurium have been commonly isolated from fresh vegetables (Rana et al., 2010; Kisluk et al., 2012).

Salmonella spp. can be transferred to the food chain directly from human or animal faecal sources, run-off of nearby farms, untreated manure (Islam et al., 2004), or from contaminated irrigation water (Kroupitski et al., 2009). Additionally, various routes for cross-contamination in the kitchen and processing environments, where mishandling practices and improper hygienic practices are prevalent, have been reported to contribute significantly in the transmission of foodborne pathogens to food (Chen et al., 2001; Kusumaningrum et al., 2004; Luber et al., 2006). More specifically, the transmission of pathogens to food is often facilitated by poor personal hygiene of food handlers, inadequate storage or processing food on equipment, and contact surfaces that were not properly cleaned and disinfected (de Jong et al., 2008). Of food contact surfaces, cutting boards were shown to represent critical risk factors of cross-contamination and recontamination events (Redmond & Griffith, 2003; Van

Asselt et al., 2008; Tang et al., 2011), as constant sources of pathogens to food (Chen et al., 2001; Moore et al., 2003; Cliver, 2006).

Chapters 4-5 showed that the majority of small restaurants used plastic cutting boards, half of which relied on washing in water with or without soap with no sanitizer used thereafter. The use of plastic cutting boards have gained popularity in the last two decades with the introduction of plastic cutting boards in the 1970s in replacement of wooden ones for reducing the risk of cross-contamination particularly from remaining juices of raw meat and poultry on the deep cracks on the surface of the board that provides a suitable environment and source of microorganisms to be transmitted to other foods on the same surface (Gough & Dodd, 1998). However, there is a clear evidence that when plastic cutting boards are inadequately cleaned such as after cutting raw meat and poultry they can harbour pathogenic microorganisms leading to hazardous events, even so in some circumstances with one single bacteria adhered to the surface (Ravishankar et al., 2010; Soares et al., 2012b).

Therefore, in conditions of hygiene failures in restaurants or home settings, remaining pathogens, including *Salmonella* populations, are able to attach to plastic cutting boards and other various types of food preparation surfaces in the food processing environment and multiply in favourable environments (Scott & Bloomfield, 1990, 1993; Frank, 2001; Bae et al., 2012;). Those colonized cells are also capable of forming biofilms which shall potentially act as a continuous source of post-processing bacterial contamination posing significant health hazards (Stepanović et al., 2004; Oliveira et al., 2006; Pui et al., 2011). Back in 1998, almost half of the reported cases of foodborne outbreaks in France were related to contamination by equipment with biofilms (Haeghebaert et al., 2001).

As bacteria are dislodged from biofilm formed on contact surfaces, they have the propensity to attach to food surfaces and to transfer to other food, resulting in foodborne illnesses (Pui et al, 2011).

Many reports have focused on survival and transfer of pathogens including *S*. Typhimurium from food of animal origins to surfaces or other food types in meat preparation (Gough & Dodd, 1998; Kusumaningrum et al., 2004; Moore et al., 2007; Ravishankar et al., 2010). Different factors influencing the attachment capacity of *Salmonella* spp. have been suggested; for instance, Pui et al. (2011) indicated that attachment is strongly strain-dependent; others pointed out that the rates of transfer of *Salmonella* cells between various types of surfaces can be affected by the type of bacteria and the moisture levels on surface, type of contact surfaces (Milling et al., 2005), inoculum size (Montville & Schaffner, 2003) and conditions of the source and destination (Sattar et al., 2001; Gill & Jones, 2002; Goh et al., 2014).

Although much research has shown that cross-contamination can occur between food contact surfaces and foods, the studies mostly focused on bacterial residence time, types of equipment surfaces and other conditions, typically using a single food being sliced (one-time food-slicing scenarios). Limited information exists on cross-contamination from foods of plant origin (Wachtel & Charkowski, 2002), and the order of magnitude or trend in cross-contamination of the same type of food sliced subsequently on same contaminated surface.

Only recently, Zilelidou et al. (2015) described the bacterial transfer during consecutive knives cutting of lettuce leaves and its distribution between cutting knives and lettuce. In many Mediterranean and Middle Eastern countries, parsley is typically eaten raw and prepared by finely chopping several batches of the leaves for processing into appetizers, RTE salads, and garnishes served in food service and home settings. Because of the convoluted nature of parsley leaves and no precedent for transfer studies with this vegetable, hypothetically some variability in the transfer rate will occur (Rodríguez et al., 2011; Zilelidou et al., 2015). Therefore, in this study parsley was chosen to evaluate the transfer rate of S. Typhimurium in scenarios that resemble normally occurring operations in restaurants and home kitchens. The aim was to quantify the transfer rate of *Salmonella* across all chopped batches from originally contaminated parsley. The transfer rates would be hypothetically lower upon consecutive chopping of each new batch of parsley on the same contaminated surface. The data of this study could be useful in quantitative microbial risk assessment of *S.* Typhimurium on parsley under different food handling conditions and as a model to other leafy greens.

8.2. Materials and Methods

8.2.1. Strains and cells suspension preparation

S. Typhimurium LT2 was adapted to grow in the presence of 50 mg/ml nalidixic acid (Sigma-Aldrich), through stepwise exposure to nalidixic acid (Bio-rad laboratories Ltd, Hemel Hempstead, UK), i.e., colonies of *S.* Typhimurium were suspended in TSB containing 10, 20, 30, 40 and 50 µg/ml (Parnell et al., 2005). A loopful of culture was taken from the highest concentration and streaked onto plate count agar (Bio-rad laboratories Ltd, Hemel Hempstead, UK) supplemented with 50 µg/ml nalidixic acid (PCAN) and incubated at 37ºC for 24 h. One to two colonies of this strain were grown overnight at 37°C in 10 ml of tryptic soy broth (TSB; Conda, Spain) supplemented with 50 µg/ml nalidixic acid (TSBN), and incubated at 37 ºC for 18-20 h to yield 10^9CFU/ml which was initially verified by direct plating. Target concentrations of approximately 3 and 6 log CFU/g were prepared by suspending 1 ml of an overnight culture of appropriate dilution in 1 l of 0.1% (PW).

8.2.2. Contamination of parsley

Bundles of fresh parsley were purchased from a local grocer on the day of the experiment. They were washed with running tap water for 10 s to remove dirt and soils. Only green fresh leaves with 5 cm stalks (100g) were used and inoculated by dipping into inoculum at the target concentrations of *S*. Typhimurium for 30 min. to allow attachment. Thereafter, inoculated parsley leaves were placed on sterile papers in a laminar airflow and dried for approximately 60 min. (Ruiz-Cruz et al., 2007).

The immersion process of fresh produce is a possible point of contamination at post-harvest based on the observations during post-harvest washing (Section 3.4.1); hence, dip-inoculation was considered to be a suitable method for simulating contamination in commercial fresh produce operations (Beuchat et al., 2001).

8.2.3. Cutting boards preparation and cross -contamination scenarios

Polyethylene domestic cutting boards (CB) were purchased from a local kitchenware store and were disinfected by soaking in 0.30% sodium hypochlorite (Clorox® Bleach) overnight before use. Cutting board surfaces were thoroughly rinsed by immersion in hot sterile water (ca.80ºC) to remove any remaining disinfectant prior to use (Pui et al., 2011) then soaked in 70% ethanol for 1h and air-dried in the laminar flow cabinet prior to use in each experiment (Kusumaningrum et al., 2003). The cleaning process was validated by a swab test, which confirmed the absence of *S.* Typhimurium after each experiment.

The different scenarios performed in the laboratory experiments were designed to determine 1) the transfer rate of *S*. Typhimurium each time a new batch is chopped after one contaminated bundle of parsley that typically weighs 100g, and 2) to quantify the remaining cells on the cutting board each time we chopped a new batch, as follow:

Scenario 1 (CB Instant): Inoculated parsley (100g) was initially chopped on a clean disinfected CB. Afterwards, 5 batches of ca.40g parsley (the quantity a hand may grab tightly within the defined chopping area) were chopped consecutively and instantly on the same cutting board.

The size of consecutive batches was set to 40g for all scenarios to ensure consistency in the experiments. This was the quantity a hand grabbed fully within the defined area of the CB, but few leaves remained adhered on the board after chopping.

Scenario 2 (CB WW): After initially chopping 100g of inoculated parsley, the cutting board was placed at room temperature (21-22°C) for 1h with some remaining exudates and leaves to mimic busy food operations and intermittent chopping practices; then the CB was rinsed under running tap water for 5 s to remove any adhering leaves and stored at 30ºC for 24 h, a typical holding temperature in small eateries during summer. The next day, clean batches of parsley (30-40g) were instantly chopped on that same CB.

Scenario 3 (CB SW): Similar to Scenario 2 except that water washing was combined with three manual scrubbings in one direction along the defined chopping area using a soft sponge containing kitchen soap detergent (15-20% anions surfactants). Overall contact time was estimated to be about 10 seconds, although sometimes this was shorter during peak food preparation occasions. The sponge was disinfected before use in replicated experiments by soaking in 0.30% hypochlorite for 5 min, followed by thorough rinse in hot water (ca.80 °C) and air drying.

Scenario 4 (Changing gloves): This scenario was designed to observe variation in cross-contamination rates which could be attributed to a person's hand coming in contact with contaminated surfaces and later to un-inoculated batches during chopping. For this, gloves were regularly replaced by a fresh sterile pair before holding and chopping each clean batch. A total of 6 batches, with similar weighs as in previous scenarios, were chopped in succession and analyzed in triplicates., to allow observation in trend differences.

To determine the number of parsley bundles to chop, the inoculum level was considered in context, with the previous observations in the SMEs and understanding of home kitchens practice. Thus, up to 6 bundles (n=6) would cover what a small restaurant may prepare for the day, even when it likely exceeds what is prepared in home kitchens. At low inoculum levels, the number of bundles was reduced to 3 for observations of bacterial transfer and for microbial detection.

Experiments with low inoculum levels were conducted in two scenarios only, CB Instant and CB WW, for comparison with high inoculum size. All experiments were repeated 3-5 times, except for Scenario 4 which was conducted once as an additional validation step. The values represent the means of replicated experiments.

To remove any potential effect of utensil characteristics and transfer, autoclaved scalpels instead of knives were used for chopping. The same scalpel and hand gloves were used in each experiment to mimic what typically occurs in food services. A standard fixed duration for chopping each batch of parsley was 1 min over a 21 cm^2 area.

8.2.4. Detection and enumeration of *S*. Typhimurium

For detection of direct presence of *Salmonella* spp. non-selective and selective enrichment steps were performed according to ISO 16140. Parsley samples that had not been inoculated (control) were confirmed for the absence of *Salmonella*.

Enumeration of *S*. Typhimurium was determined on each triplicate of chopped batch of parsley. From each, 10g were individually weighed, homogenised in TSB and stomached for 2 min at 230 rpm. Aliquots of 100 µl were spread-plated in duplicate onto Rapid'*Salmonella* agar (Bio-rad laboratories Ltd, Hemel Hempstead, UK) supplemented with nalidixic acid and incubated at 37º C for 24 h. To avoid over or underestimation of counts, average detection limit was set to 0.7 log CFU/g for <10 CFU/g - enumerated on the lowest dilution and when detection results are positive.

8.2.5. Procedures for the recovery of *S*. Typhimurium cells from CBs after initial contaminated parsley and subsequent chopping

An initial swabbing of the CB was performed after the initial chopping of 100 g contaminated parsley, and after chopping each new batch of uncontaminated parsley.
CB was divided, by marking, into 6 sections of 7cm x 3 cm area for experiments with high inoculum levels, and 7 sections areas for the low inoculum levels in view of the lower number of batches to chop, hence allowing a swab of 2 separate sections per each batch (replicates). The partitioning of CB was made to avoid swabbing the same site more than once during 6 successive chopping, hence avoiding errors of underestimating the actual numbers of cells remaining on CB after each new single batch. Sections were swabbed with cotton-tips moistened in BPW (Bio-rad laboratories Ltd, Hemel Hempstead, UK) in three different directions: left to right, top to bottom, and diagonal (Pui et al., 2011). The last swab of the 6th section was taken over the entire area (126 cm^2) after the final batch. For the low inoculum level, the very first section was swabbed twice, at the initial and last stage (also after the last batch).

Each swab was placed into a tube with 9 ml BPW and vortexed vigorously for 1 min. and 100 µl of the tenfold serial dilutions were spread-plated on duplicate plates of Rapid' *Salmonella* agar supplemented with nalidixic acid and incubated for 24 h at 37 ºC. Counts were expressed as log CFU/cm^2, calculated according to the formula:

$$\frac{\text{Average log10 CFU/plate x (volume of original suspension)}}{\text{(Total surface area x 1 swab) (dilution factor) (volume applied on plate)}}$$

8.2.6. Data presentation

The quantitative data was expressed as means ± standard deviation. To determine the trend of cross-contamination and transfer rate of *S.* Typhimurium (Tr) from the origin of contamination, i.e., one contaminated chopped bundle of parsley to each consecutively chopped clean batch, the Tr was estimated by dividing CFU on non-inoculated samples (receiver) with CFU on inoculated samples chopped on the same cutting board surface (Chen et al., 2001; Pérez-Rodríguez et al., 2008; Goh et al., 2014), i.e., [(CFU on the clean parsley (recipient) / CFU on contaminated parsley(donor)]. Transfer rates are multiplied by 100 to be presented as % Transfer rate. Additionally, the Tr data were log10 transformed, i.e., (log10 ratio of [CFU/g (receiver)/CFU/g (donor)] for easier understanding and presentation at top scale (as log reduction) (Chen et al., 2001).

8.3. Statistical analysis

All statistical analysis, distributions and data presentation in frequency histograms of transfer rates and log CFU/g were performed using the IBM SPSS version 22.

Differences between distributions as affected by different handling conditions of CBs were determined using a one-sided Wilcoxon's matched pairs signed rank test for two related groups and Friedman test for more than two related groups on the same continuous, dependent variable.

Statistical significance among mean values of log CFU/g of *S*. Typhimurium for the different batches chopped on same cutting board was determined using Kruskal Wallis test. Mann-Whitney U test was performed to determine the statistical differences between means distribution of the transfer rates between high and low inoculum levels. Spearman's rho correlation was performed to determine association between inoculum size and transfer rate of *S.* Typhimurium to parsley samples. Statistically significant difference was set at $p < 0.05$.

8.4. Results

8.4.1. Overall Tr of *S*. Typhimurium to parsley

Results of transmission of *S*. Typhimurium populations from artificially contaminated parsley to all processed uncontaminated batches via cutting boards are presented in Table 8.1.

After chopping parsley inoculated at low concentration levels with *S*. Typhimurium, the recovered cells on uninoculated samples instantly chopped on the same surface (CB Instant) ranged from 2.00 to 3.85 log CFU/g and mean value was significantly higher than on samples chopped on the CB held for 24 h at 30ºC after a water wash (CB WW) ($p < 0.05$) (Table 8.1).

Table 8.1. *S*. Typhimurium transferred to uninoculated parsley chopped subsequently to inoculated batches

Inoculum level	Cutting board	Initial inoculated batch‡	N†	Mean log CFU/g (min.-max.)	Median log CFU/g	Percent transfer rate, (min.-max.)
Low	CB Instant	3.30	64	2.94a (2.00 - 3.85)	2.87	60a (2.00-100.00)
	CB WW	3.20	55	2.67b (2.00 - 3.48)	2.70	64a (2.00- 100.00)
High	CB Instant	6.08	50	3.41 (<1.00†- 5.51)	3.54	1.2^{b} (0.01-25.00)
	CB WW	5.95	15	3.67 (2.78 - 4.69)	3.64	1.4^{b} (0.05-7.50)
	CB SW*	6.23	26	3.50 (<1.00- 4.72)	4.07	1.0 (0.05-2.83)

‡The inoculated parsley (100g) was initially chopped as the very first batch. Low inoculum level range= 2.85 - 4.00 log CFU/g. High inoculum range= 5.80 – 6.32 log CFU/g
† The number of analysed samples of parsley
*CB SW scenario was not tested with low inoculum levels
Different superscript letters in the same column indicate significant difference at $p < 0.05$

Wilcoxon Signed Rank test showed that the median of differences of recovered *S*. Typhimurium was significantly different between CB Instant and CB WW ($p < 0.001$). The Tr of bacterial cells to parsley chopped on CB Instant and CB WW recorded high mean values, 0.60 ± 0.65(60.0%) and 0.64 ±0.46 (64.0%) with a Tr magnitude ranging from 2-

100% (Table 8.1), respectively. Conversely, at high inoculum level, Tr data did not differ significantly among the three cutting boards scenarios ($p>0.05$). The concentration of *S.* Typhimurium cells ranged from <1.00 on CB Instant and CB SW, to a maximum of 5.51, 4.69 and 4.72 log CFU/g on CB Instant, CB WW and CB SW, respectively (Table 8.1) as a result of a substantially lower cross-contamination rate. Bacterial Tr to parsley were highly variable being as low as low as 0.01 to as high as 25.00% via CB Instant. Washing CBs with soapy water combined with sponge scrubbing did not effectively reduce the bacterial transfer to parsley although maximum values diminished to 7.50% and 2.83% via CB WW and CB SW, respectively (Table 8.1). Statistical analysis showed that Tr of *S.* Typhimurium to uninoculated parsley was significantly higher with the initial chopped samples (source) inoculated with low levels than with high contamination levels ($p < 0.05$), on both, CB Instant and CB WW. The CB SW scenario was not tested at low inoculum level (Table 8.1). Spearman's rank-order confirmed that the Tr is significantly and negatively correlated with the contamination level at source. ($p < 0.001$). However, the correlation was stronger for CB Instant ($r_s = -0.846$, n=110) than for washed CB (CB WW, $r_s = -0.676$, n=68).

The frequency histograms at a logarithmic scale in Figure 8.1 and Figure 8.2 represent merged data of all batches processed in each cutting board to examine the general cross-contamination events when all batches would be processed and mixed together. The log reduction extended with a high frequency from 0.57 to -1.7 log Tr on CB Instant, and slightly broader to -2.00 on CB WW. The value “zero” represents the limit of the transfer rate, i.e., Tr=1 (100%); values above 0 were encountered in some samples when the recovered population on parsley was higher than the averaged values of concentrations originally on contaminated parsley (CFU) i.e., the denominator of the Tr fraction. This was similarly encountered by Zilelidou et al. (2015) as they reported a high variability in log Tr for *L.*

monocytogenes from knife to lettuce ranging between -1.0 and -0.5 on day 0 with log reduction reaching in some occasions below -1.00 log CFU during the first cuts.

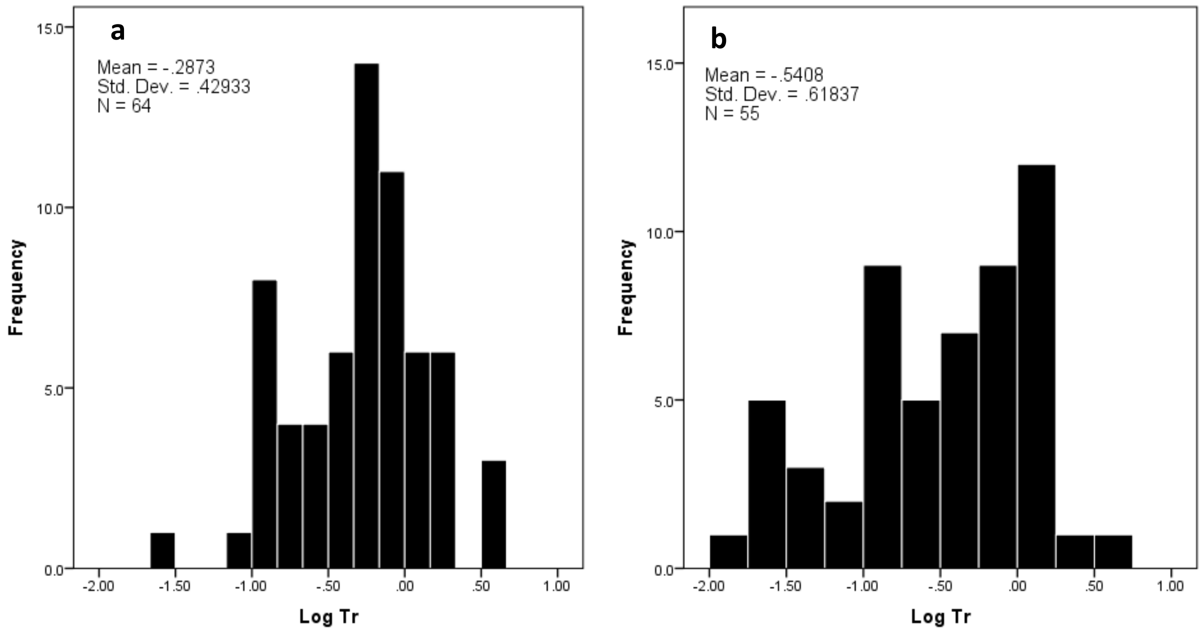


Figure 8.1. Frequency of distribution of log Tr of *S*. Typhimurium on all uninoculated chopped batches

A comparison of log Tr of *S*. Typhimurium from inoculated parsley to uninoculated batches chopped (merged data, a) instantly on same cutting board (CB Instant), b) after simple water wash of same cutting board and 24 h holding at 30ºC (CB WW), subsequent to initially chopped 100g parsley inoculated with, a) 3.26 log CFU and b) 3.16 log CFU.

At high inoculum level, the range of log Tr data shifted higher the scale (<0.001) than that observed at low inoculum level (Figure 8.2) due to a lower cross-contamination rate. Despite the application of water (CB WW) and soapy water coupled with scrubbing (CB SW), the distribution of data didn't differ greatly (p > 0.05), as aforementioned.

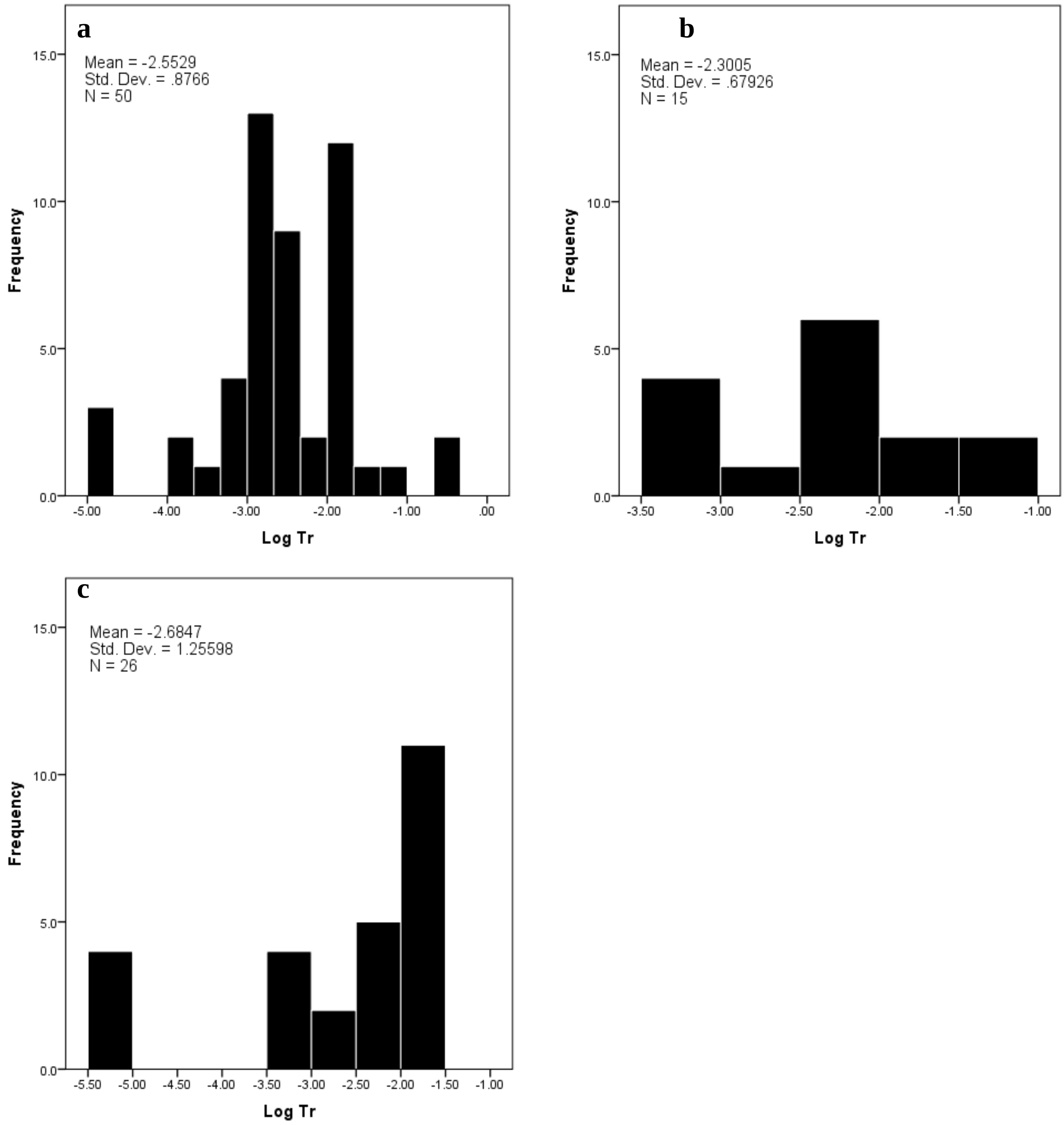


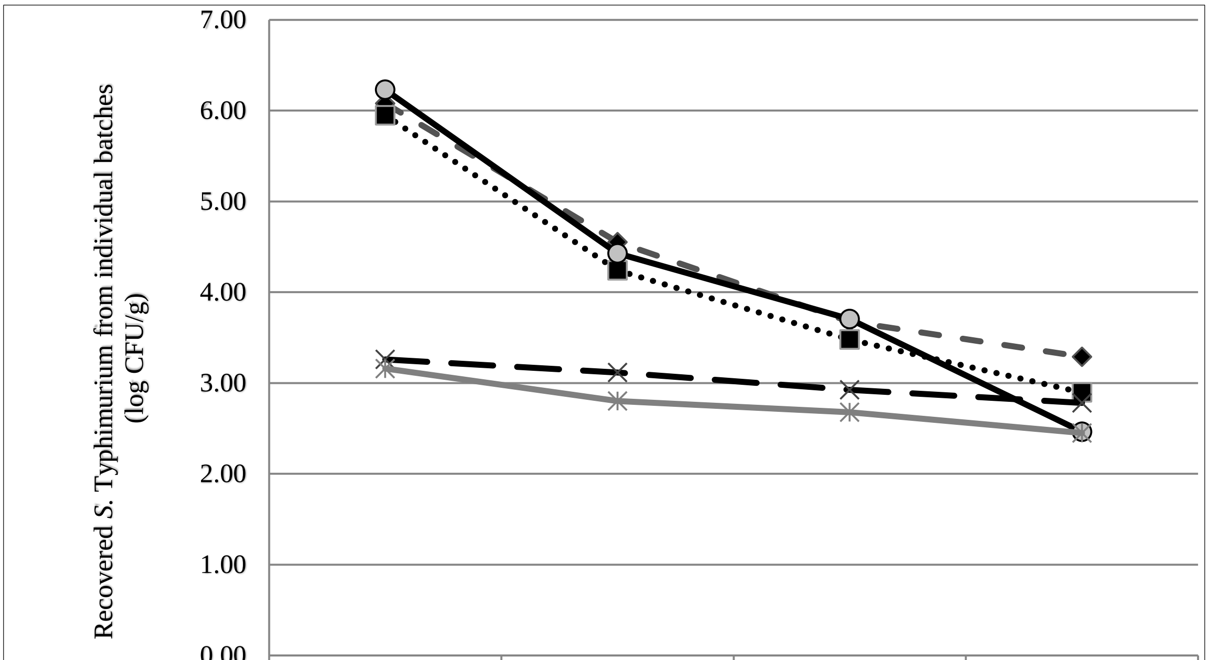
Figure 8.2. Frequency of distribution of log Tr of *S*. Typhimurium on all uninoculated chopped parsley

A comparison in log Tr of *S*. Typhimurium to uninoculated parsley chopped, a) instantly on same cutting board (CB Instant), b) after water wash of same cutting board and holding for 24 h at 30ºC (CB WW), c) after soap and water wash combined with soft sponge rubbing and holding for 24 h at 30ºC (CB SW), subsequent to initially chopped 100g parsley inoculated with high inoculum level of, a) 6.08, b) 5.95 and c) 6.23 log CFU/g.
The outliers' data values >-4.00 represents samples with bacterial counts below detection levels (10 CFU) (n=8)

8.4.2. Distribution data of *S*. Typhimurium to individual batches of parsley chopped consecutively on the same surface

Patterns in log reduction histograms at batch levels in relation to different CBs paralleled those observed with merged data; for instance, at low inoculum levels, log Tr data were generally distributed from 0.57 to -0.90 on the first batch (B1) chopped on CB Instant; cross-contamination was significantly reduced by the third (B3) as the distribution extended further down to -1.00 and more than -1.50 log reduction ($p < 0.05$) (Figure 8.1). There was a significant reduction in mean values of recovered bacterial cells from B1 to B3, 3.12±0.50, and 2.78 ±0.60, respectively (Figure 8.3); on the contrary, the Tr was similar across all batches when the CB was washed with water ($p > 0.05$) as shown in the mean counts of 2.80 ±0.22 and 2.47 ±0.37 log CFU/g recovered from B1 and B3, respectively (Figure). This explained the significant differences in Tr and log CFU values between CBs when data of all batches were merged (Table 8.1).

At high inoculum level, Tr of *S*. Typhimurium was significantly the highest to B1, and considerably dropped by the third batch on CB Instant and CB WW ($p < 0.05$); however, the distribution of bacterial cells was similar across all batches chopped on CB SW ($p > 0.05$) (Table 8.2, Figure 8.4).



	Inoculated batch*	B1	B2	B3
CB instant-H	6.08	4.55	3.68	3.29
CB WW-H	5.95	4.24	3.48	2.90
CB SW-H	6.23	4.43	3.70	2.46
CB instant-L	3.26	3.12	2.93	2.78
CB WW-L	3.16	2.80	2.68	2.45

Figure 8.3. The population size of *S*. Typhimurium on successively chopped batches of parsley after initial chopping of artificially contaminated samples

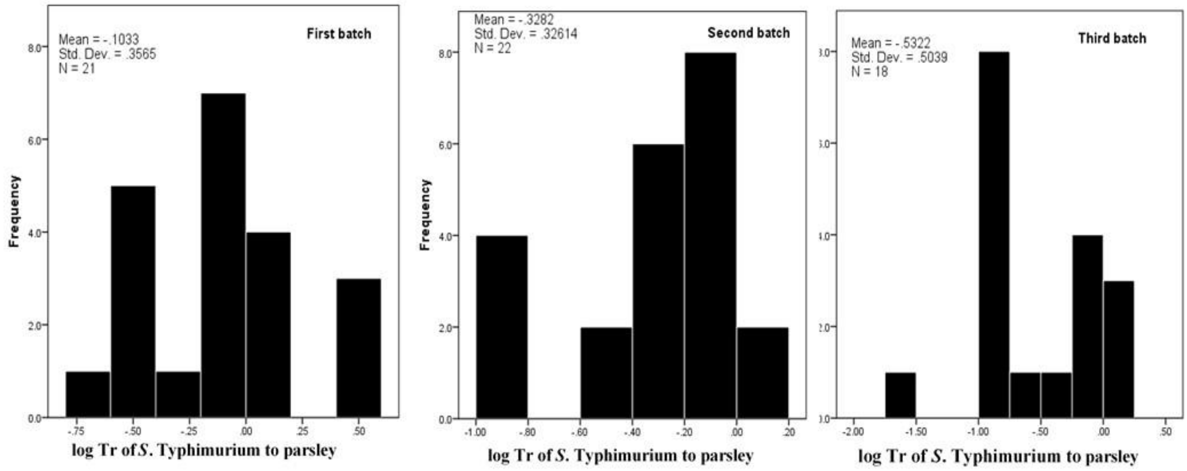
A decreasing trend in the recovery of *S*. Typhimurium along the batches of uninoculated parsley chopped, subsequent to initially inoculated 100g of parsley, a) instantly on same cutting board (CB Instant), b) after water wash the CB and holding for 24 h at 30ºC (CB WW), c) after washing the CB with soapy water and soft sponge rubbing and holding for 24 h at 30ºC (CB SW). The latter scenario was not tested with low inoculum levels.
*Initially chopped inoculated parsley (log CFU/g)
L = low inoculum; H= High inoculum.

Table 8.2. Tr of *S*. Typhimurium to three consecutively chopped uninoculated parsley subsequent to contaminated samples on same cutting board surface

	Parsley batches % Tr, (min-max)					
	Low inoculum			High inoculum		
Cutting board handling	B1	B2	B3	B1	B2	B3
CB Instant	81.0[a] (23.0-100)	58.5 (10.0-100)	53.1[b] (2.0-100)	5.5[a] (0.76-25)	0.7[b] (0.09-2.0)	0.5[b] (0.09-1.0)
CB WW	61.0 (9.0-100)	55.0 (3.0-100)	30.0 (2.0-100)	3.2[a] (0.06-7.5)	0.4 (0.08-0.7)	0.08[b] (0.05-0.08)

Different superscript letters in the same row at each inoculum level indicate significant difference at $p < 0.05$
B1=first batch; B2=second batch; B3=third batch

a. CB Instant



b. CB WW

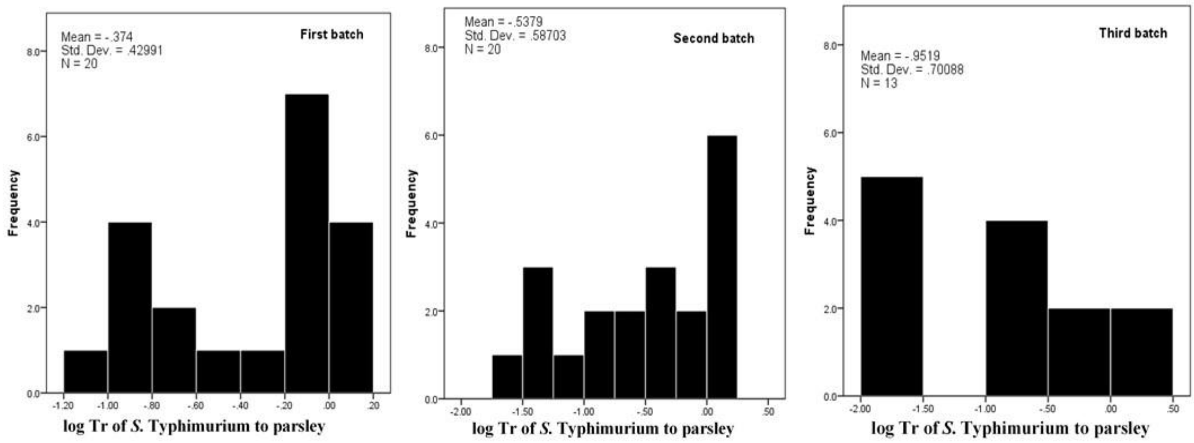
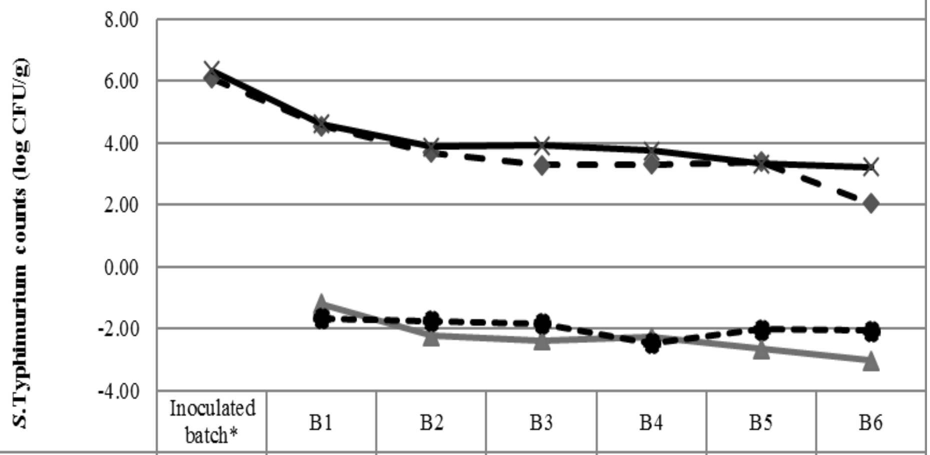


Figure 8.4. Frequency of distribution of log Tr of *S*. Typhimurium on individual chopped batch of parsley
Comparison of log Tr. of *S*. Typhimurium from initially chopped inoculated parsley (100g) with ca. 3 log CFU/g to three consecutively chopped batches of uninoculated parsley (30g) on, a) CB Instant and b) CB WW.

The results in Tr were negligible when the hand gloves were regularly changed with the chopping of each uninoculated batch (Figure 8.5). In both scenarios (1 and 4), Tr to B1 was constantly higher than to all successive batches (p < 0.0.5) with a remarkable difference at the last batch, B6. This may imply that although changing gloves still have contributed to pathogen transmission onto the last batches, the contaminated surface of the CB is relatively the key contributing factor to a constant transmission of pathogens to all parsley batches.



	Inoculated batch*	B1	B2	B3	B4	B5	B6
CB Instant (log CFU/g)	6.08	4.55	3.68	3.29	3.31	3.38	2.03
CB Instant (log Tr)		-1.21	-2.25	-2.38	-2.28	-2.66	-3.03
CB Instant-gloves changed (log CFU/g)	6.34	4.60	3.87	3.92	3.76	3.32	3.22
CB Instant gloves changed (log Tr)		-1.68	-1.74	-1.83	-2.47	-2.04	-2.06

Figure 8.5. Log Tr and *S*. Typhimurium counts recovered from consecutively chopped batches of parsley on CB Instant with and with no changing gloves

The reduction pattern in cells recovery and log Tr of *S*. Typhimurium was consistent through the first five batches of parsley chopped instantly subsequent to inoculated samples (CB Instant), with and without changing gloves. B1 to B6 is the order of chopping order of each batch following the first 100g inoculated samples
*Initially chopped inoculated parsley (log CFU/g)

8.4.3. Recovery of *S*. Typhimurium cells from the cutting board surfaces

Table 8.3 and Table 8.4 show the numbers of *S.* Typhimurium recovered from CBs after the initial chopping of inoculated samples (S0) and after each subsequent chopping of a new batch (S1-S6).

At high inoculum level, the mean values of recovered *S.* Typhimurium (log CFU/cm^2) showed a decreasing trend as more batches were sequentially placed on the same surface.

Overall, the number of recovered cells ranged from below detection limit (10 CFU/g), observed towards the last chopped batches, to a maximum mean of 0.23 log CFU/cm^2 at the early stage of chopping, which is equivalent to a maximum of 4.14 log CFU/swabbed area. Whereas at low inoculum levels, fewer organisms were recovered from CB Instant (0.10-0.12 log CFU/cm^2) ranging from below detection limit to 0.17 log CFU/cm^2. Washing CB with water significantly decreased the number of microorganisms on CBs to mean values of 0.09 to 0.11 log CFU/cm^2 ($p < 0.05$) with a maximum level of 2.73 log CFU/swabbed area. Mann-

Whitney U test indicated a significantly lower level recovered from CB Instant exposed to low inoculum levels than from that exposed to high inoculum levels.

Table 8.3. Recovery of *S.* Typhimurium from CB Instant after consecutively chopped batches of parsley subsequent to inoculated sample with high inoculum levels

Sequence of swabs†	N	Mean log CFU/ cm^2 (min.-max.)
S0	3	0.22 (0.18 - 0.25)
S1	3	0.23 (0.20 - 0.24)
S2	3	0.21 (0.16 - 0.25)
S3	3	0.20 (0.16 - 0.23)
S4	3	0.14 (<1.0 - 0.15)
S5	3	0.16 (<1.0 - 0.20)
S6	3	0.03 (<1.0 - 0.03)

†Swabbing after chopping parsley. S0 is the first swab taken after initial chopping of 100g parsley inoculated with a mean population size of 6.13 log CFU/g, followed by 6 swabs (S1-S6), each taken after chopping uninoculated batch (for the additional 6 batches).

Table 8.4. Recovery of *S.* Typhimurium from CB Instant after consecutively chopped batches of parsley subsequent to inoculated sample with low inoculum levels

Sequence of swabs†	CB Instant		CB WW	
	N	Mean log CFU/cm^2 (min.-max.)	N	Mean log CFU/cm^2 (min.-max.)
S0[a]	12	0.10 (<1 - 0.17)	10	0.09 (0.09 – 0.09)
S1	12	0.12 (<1 - 0.14)	10	0.10 (<1– 0.11)
S2	12	0.12 (<1- 0.14)	10	0.11 (<1– 0.12)
S3	12	0.12 (<1- 0.14)	10	0.11 (<1– 0.13)
S4	6	0.11 (<1- 0.12)	5	0.10 (<1– 0.12)

† Swabbing after chopping parsley. S0 is the first swab taken after initial chopping of 100g parsley inoculated with a mean population size of 3.73 and 3.15 log CFU/g for CB Instant and CB WW, respectively, followed by 3 swabs (S1-S3), each taken after chopping un-inoculated batch (for the additional 3 batches). S4 = the last swab sampled from the same S0 area at the end of chopping process.
In the case of CB WW, the swab S0 was taken at 24 h after water wash and incubation at 30ºC.

8.5. Discussion

The results of this study corroborate with several studies that demonstrated an inverse relationship between inoculum size and Tr of pathogens (Montville & Schaffner, 2003); in this context, the results concur with Ravishankar et al. (2010) who reported a high Tr of 75% as a result of a low inoculum size on the CB surfaces and knives. Likewise, Fravalo et al. (2009) observed that the percent Tr of *Campylobacter* from contaminated chicken to cutting boards was inversely related to the initial inoculum level.

While the precise mechanism underlying this relationship is not well established, Montville and Schaffner (2003) suggested that the reduction in Tr at high inoculum level could be attributed to enhancement in cell adherence to the donor surface when bacterial concentrations are high, inferring from findings of Takeuchi and Frank (2000) who highlighted the improved attachment of *E. coli* O157:H7 to lettuce leaves due to higher inoculum levels.

Results of the present study were also consistent with a study by Pérez-Rodríguez et al. (2011) where a risk mathematical model showed *E. coli* O157:H7 was able to survive and contaminate final bags of fresh-cut lettuce in all simulated interventions scenarios. They are also consistent with a study by Soares et al. (2012b) where an average of 2.71 log CFU was recovered from tomatoes cross-contaminated via cutting boards that were formerly contaminated with artificially inoculated chicken skin with *S*. Enteritidis (5 log CFU/g). Several studies examining bacterial Tr between surfaces and single sliced foods reported a substantially high variability in data; Chen et al. (2001) recorded a Tr of *E. aerogenes* between various surfaces ranging from 0.0005% to 100%, more notably, between clean hands previously contaminated with 10^6 cells and lettuce (0.003 to 100%); this variable pattern was observed from individual to individual despite that all participants followed the same experimental protocol. In comparison to above studies, Tr values to single sliced batch (B1) was generally lower and also varied, although to a lesser extent, from 0.76 to 25% on CB

Instant and 0.06-7.5% on CB WW (Table 8.1). This difference and variations in Tr data are likely to be attributed to inner folds of parsley leaves, the level of *S.* Typhimurium originally on parsley, and experiment set-ups. In other studies, bacterial transmissions occurred from inoculated abiotic to biotic surfaces, whereas in the present work, cross-contamination events were studied from contaminated parsley to clean uncontaminated batches by means of CB. In this case, heterogeneity in attachment levels of cells to CBs could occur as affected by angle of contact on CB, thus the variations in Tr to batches of uninoculated leaves. Nevertheless, the scenarios in the present work may closely reflect the real and natural variability expected among individuals during chopping parsley in restaurants and homes settings.

Moore et al. (2003) also reported a wide range in Tr data from stainless steel surfaces to one-time sliced lettuce for *S*. Typhimurium, 13.15-67.63% and *Campylobacter*, 0.19-43.97%.

It is maintained that large variations in Tr data are a consequence to errors inherent to microbial collection from surfaces (Carrasco et al., 2012), methodological differences and difficulty in controlling all factors involved in bacterial transfer phenomena which in this case would not allow for easy comparisons among different cross contamination studies (Zilelidou et al., 2015).

In general, *S*. Typhimurium was apparently readily transferred into cutting boards, and later was capable of contaminating chopped parsley both at instant contact and at 24 h after washing, with the ability to cross-contaminate the entire batches of leafy greens most outstandingly at low contamination level.

The survival of bacteria for long time on surfaces is documented in various citations where bacterial counts increased over time and wet surfaces played an important role in bacterial transfer to food (Scott et al., 1990). Many other factors were suggested to influence pathogens transmissions between surfaces such as the topography of different kind of cutting boards (Goh et al., 2014) and temperature of food (Goh et al., 2014; Tang et al., 2011). Nonetheless, the survival of *S*. Typhimurium for prolonged time (24 h) has been probably sustained by remaining substrates from parsley juice within knives-scars and fissures on the plastic boards surfaces which have been shown to be very difficult to clean and disinfect, although this may vary among the types of plastic cutting boards (Cliver, 2006). It was evident in this study that the density of bacteria can remain constant up to 24 h supported by nutrients abundance (Dawson et al., 2007). Although the moisture levels on CB surfaces were not tested, CBs were apparently dried out after 24 h incubation at 30ºC, and still was found to harbour microorganisms although at constant and in other conditions at reduced levels. There is a wide recognition that *S*. Typhimurium is capable of persistent survival on dry surfaces for up to 4 weeks and that the transfer rate to food was reduced as the bacterial exposure time on the surface increased up to 24 h (Dawson et al., 2007). Other authors proposed that higher temperatures enhance the drying process resulting in a decrease of cultivable bacteria; correspondingly, higher initial moisture promotes longer drying process and thus the bacteria could survive longer on wet surfaces although the bacteria density decreased with time (Milling et al., 2005).

The plausible explanation for the reduced transfer rate observed in this study is that *S*. Typhimurium might have been stressed or injured during the washing process (Dawson et al, 2007) and more likely that there is a threshold value of cells that can be transferred depending on the capacity of cutting board surface to harbour attached cells under the conditions of the study. Thus, even if 10^6 cells are present on surface, only a magnitude of 10^3 can be transferred from the contaminated surface to fresh (uninoculated) batches as a result of simple contact.

This work paralleled several studies that revealed the inefficiency of water or water and soap in eliminating pathogens from cutting board surfaces, hence the limited reduction in

Tr (Cogan et al., 2002; Ravishankar et al., 2010; Soares et al., 2012b), and it showed that without appropriate disinfection procedures for CBs used with contaminated fresh leafy greens, the risk of cross-contamination remains regardless of the number of batches processed at one time, particularly when the infective dose can be as low as 10 cells. Results on surface swabs support those obtained on the Tr to parsley, and the assumption that with high inoculum size at the source, the adherence of bacterial cells to the surface is enhanced and vice-versa (Montville & Schaffner, 2003).

The results of this work are in accordance with other studies where bacteria were recovered from plastic cutting boards at 5 min resident drying times and 24 h following cold wash water (Abrishami et al., 1994) and where low counts (<1 log CFU/g or cm^2) of *S.* Newport remained on the plastic surface previously exposed to contaminated poultry, and tested after washing with soap, warm water, and vigorous scrubbing (Ravishankar et al., 2010).

Although some swabs had counts below detection limits, detection tests recorded a positive presence of the pathogen. As discussed earlier, *S.* Typhimurium has the propensity to survive in high levels depending on nutrient and water availability (Pui et al., 2011) within the cutting boards crevices for a prolonged period in stressful conditions. In such conditions, microorganisms may enter a state of metabolic inactivity, resulting in viable and non-cultivable cells that are able to grow again under favourable conditions (De Boer et al., 1990). There is the limitation that part of the inoculum within the knives-scarred plastic surfaces could possibly become unavailable to the swabs used to recover it. Besides, the disadvantage of cotton swabs being limited to recover 100% of the resident microorganisms is known as the pressure applied to the surface during sampling could be too light (Moore et al., 2007). Despite this limitation, the results confirmed that bacterial cells can be transmitted from contaminated leafy greens to a sterile surface and subsequently contaminating a number of individually chopped parsley portions providing valuable information and a model for future risk assessments of cross-contamination associated with the preparation of fresh parsley.

8.6. Conclusion

This study demonstrated how *S*. Typhimurium is transferred by common operations from contaminated parsley to CBs and it would subsequently re-contaminate several batches of parsley, in this case to up to six sets when chopped consecutively on the same surface. More concerning was the recovery of presumably more resilient pathogen cells from cutting boards at 24 h at 30ºC after washing. Apparently, the simple domestic methods applied in restaurants for cleaning cutting boards by using water and water with soap combined with manual scrubbing using soft sponge reduced the transfer rate to all batches of parsley chopped subsequent to the contaminated samples on the same surface, but it did not effectively eliminate the risk of cross-contamination at instant and 24 h exposure to bacteria. The results of this study also confirmed that even at low contamination levels on the source, considerable amounts of bacteria were still transferred to parsley. Therefore, the application of additional sanitation procedures such as hypochlorite are needed on cutting surfaces not only after use with raw meat and poultry, but also with fresh produce especially that parsley is not further treated and preventive measures for fresh produce safety is poor on farms and during the post-harvest wash in Lebanon. Future research on the efficiency of the FDA recommended practice for cleaning cutting boards with soap, hot water and mechanical scrubbing on *S*. Typhimurium merits an investigation.

9. General discussion and conclusion

9.1. Summary of findings and research implication

Within this research several investigations were undertaken to identify hazards and underlying risk factors that are likely to compromise the safety of RTE salads vegetables in Lebanon as the fresh produce travels from farm-to-fork.

Chapter 2 showed that the problems of food safety in Lebanon are multidisciplinary in nature and that the safety of local fresh produce in Lebanon is at risk largely due to unregulated practices and poor planning of resources directed towards leveraging the safety of fresh produce in the domestic market. Apparently, the ineffectiveness of the food safety system and the inadequate quality infrastructure are weakened by a lack of a coherent regulatory framework. Although the resources and funded projects have promoted development and growth in some specific agri-sectors (Section 2.7 and 2.8.1), considerable funds are geared towards creating an enabling environment that supports the application of stringent food safety and quality control measures on fresh produce for the integration in the global market; whereas similar extensive supports are clearly not available for a large selection of fresh vegetables including leafy greens that are marketed internally and that constitute a major food commodity in the Lebanese cuisine (Section 2.8.1).

There is an absence of food safety monitoring and control programs for the reassurance of the domestic fresh produce safety amid the dominance of funded initiatives for export markets (Section 2.4.1). Consequently, this created an environment where ignoring or evading the basic rules of food safety were visible on the sites operated for the production to local market. Two of the producers in this research owned separate post-harvest washing facilities that differed in the hygiene standards. Produce destined for export markets were handled under improved and clean conditions unlike those sold to local consumers. This was similarly reported in most MENA countries: modern and well managed postharvest handling facilities and technologies equipped with very good sanitation, temperature management, safety and quality assurance program that comply with the requirements and market regulations are normally those operating for export markets (El-Saedy et al., 2011). The combination of information in chapters 2 and 3 implied that farmers are motivated by incentives to comply with the international marketing agreements for meeting the requirements of GAPs in order to export fresh produce .The voluntary applications of standards that vary with types of markets (export or local markets) are documented (WHO, 2008); some countries did not have mandatory national GAP standards, yet reported that the growers and distributors are willing to participate in voluntary GAP programs to increase the exporting of fresh produce. Interestingly, in that report, those countries reflected on the outstanding differences between growers' practices for domestic compared to export markets as direct payments or transportation subsidies are provided to farmers on the condition that they abide to certain standards (IFAD, 2011).

On one hand, the reinforcement of voluntary adoption of GAP standard indicates recognition of the health concerns, economic impact of food safety failures and the need to apply preventive measures to better ensure the quality and safety of fresh produce (WHO, 2008). On the other hand, and based on this research, it indicates government's interests to boost trading activities by promoting fresh produce marketing which result in a lack of equity between consumers of exported Lebanese produce and local consumers. In this respect, Zurayk and Abou Ghaida (2009) stated that "*the local wholesale markets are often the dumpsters of those fruits and vegetables that failed to meet international requirements*". They pointed out that the limited bureaucratic procedures of the MoA basically translate into limited quality control over the agricultural food chain as well as traceability.

Although the information on drivers associated with farmers' compliance to safe practices is dearth in Lebanon, publicly reported information pointed at the widespread use of sewage for irrigation in Bekaa (147 farms in Bekaa) despite producers' recognition of its hazards to consumers 'health (Hamieh, 2011). It is reported that the rural / farmers' water supplies have never been supported due to limited funds directed in this area and the pre-occupation of the government with other priorities (Hamze & Abul Khoudoud, 2004). At the time of the survey, there was a widely used informal grading system among vendors in the wholesale market. Parsley and lettuce were classified as superior in terms of size. Sewage irrigated bundles of parsley or pieces of lettuce produce much larger volume, and cost higher than smaller size leaves. It can reasonably be inferred from both chapters that in view of the volume of production, ownership of washing facilities and lands, together with the exports activities of producers, there are other additional drivers for non-compliances to food safety standards than poverty and education (FAO, 2014) and access to adequate storage facilities that should be considered in future studies and in strategizing solutions in Lebanon. These include profit-driven businesses unduly influenced by a lax in control activities and ineffective legislations governing the sector. The latter is closely related to the meager political will of local authorities and policy makers to promote safe agricultural environment and food safety, and in this case fresh produce safety (Section 2.5.1 and 2.10.).

It is thus established that fresh produce such as leafy greens and other types of vegetables produced for the local market are not necessarily the primary government's concern in Lebanon. This corroborates with EFSA's report on the assertion of some developing countries that the produce safety is not a specific source of concern to them (EFSA, 2014). This can be related to the fact that there are often no reported fresh-produce-related outbreaks, most probably due to a limited disease surveillance system (Ghosn et al., 2008; EFSA, 2014), and to the rarely monitored fresh produce-related diseases in developing countries as a result of the varying recognition of its burden (WHO 2008). The outcomes of a recent joint meeting of the United Nations Economic and Social Commission for Western Asia (UNESCWA) and FAO on the setting up a regional Arab – Good Agricultural Practices Framework (Arab-GAP) revealed concerns of countries over promoting GAP in the context of strengthening trade that outweigh those for consumers' safety (ESCWA/FAO, 2016).

In chapter 3, the field work from harvest to wholesale market demonstrated the repercussion of the gaps in the national food safety control and the lack of a robust system and regulations. Critical shortfalls in GAP, poor hygiene conditions along the chain and in post-harvest washing/packing processes were observed. The crops washing process was a major source of faecal contamination of fresh produce before reaching to consumers (Section 3.4.1) (Figure 3.2 B-C). TC and *E. coli*, indicators of faecal contamination and poor hygiene mean levels, significantly increased as fresh produce travelled from fields to washing areas, 5.13 and 1.28 log CFU/g to 6.04 and 2.24 log CFU/g, respectively. The presence of pathogens, *Salmonella*, *L. monocytogenes* and *S.aureus*, in the early stages of production as well as on washed produce is a concern (Figure 3.4). At this stage, washing processes are meant to reduce dirt and soils on crops with the added benefit of reducing microbial load (Gil et al., 2009). Pathogens and other microorganisms in wash water can infiltrate the intercellular spaces through pores or scars of bruised leaves in optimum temperature conditions (FDA, 2015b.), i.e., when produce' temperature is much higher than the water temperature, the pressure difference created may be sufficient to draw water into the fruit (Harris et al., 2003). It is thus likely that pathogens present on freshly harvested crops can accumulate in water ponds and present a risk of cross-contamination, consequently contaminating other batches of produce washed in the same ponds. Therefore, the disinfection of washing water is important to reduce the risk where the edible portions of the crop represent the highest risk when sprayed prior to harvest and with direct application of fertilizers (EFSA, 2014).

Although *S. aureus* is recognized as a poor competitor that grows poorly when present in complex microbial ecosystems as it can be inhibited or overgrown by other organisms present in food (Jay, 2000), the samples of fresh produce have shown unexpectedly high levels of *S. aureus*, up to 5 log CFU/g, and was the highest on samples from fields (Section 3.5.2) and were not significantly reduced at the post-harvest washing stage. This research suggested that *S. aureus* is potentially a principal pathogen of concern on fresh produce in Lebanon unlike studies of vegetables in western countries. The data were consistent with AL-Jaboobi et al. (2013) who reported *S. aureus* ≥5 log CFU/g from vegetables irrigated with untreated waste water and polluted river water in Yemen. Along these lines, *S*. aureus was also abundant in chicken litter (6.7-7.8 log CFU/g) (Hashem et al., 2013). The survival of pathogens on crops surfaces varies with the type of produce, pathogen and with the growing environment (Islam et al., 2004; FDA, 2015b; Kisluk et al., 2012); hence, in the light of the local agricultural conditions and common use of untreated manure, there is a need for future research to confirm the sources of *S. aureus* and its association with the use of sewage and chicken litter (Section 3.4.1). The fitness of this pathogen to survive the local conditions and agricultural production environment in relation to pre-harvest mode and time of irrigation needs to be also examined. It is well documented that animal faeces shed potentially harmful enteropathogens that can be transmitted to fresh produce by handling contaminated mud in fields or ingestion of produce grown in manures or slurries which contain harmful pathogens, such as Verocytoxigenic *E. coli* (VTEC). Therefore, *E. coli* isolates obtained of this research were further studied and were found to belong to the classical EHEC/EPEC O:H serotypes. (Faour-Klingbeil et al., 2016). Sixty percent of *E. coli* isolates including those isolated from the post-harvest area were multi-drug resistant to commonly used antibiotics in chicken and animal husbandries, indicating the pressures of practices in the agriculture production environment and the quality of water as affected by sewage or manure run-off.

WHO emphasized that irrigation water safety should be based upon risk assessment and that water guidelines in advanced economies should rely on in-country standard (WHO, 2010b). In the Leafy Green annex of the Code of hygienic practice for fresh fruit and vegetables-CAC/RCP 53–2003, Codex Alimentarius provided general recommendations stating that water that comes into "*substantial contact with the edible portion of the leafy vegetable should meet the standards for potable or clean water*" (WHO/FAO, 2007), i.e., "*which meets the quality standards of drinking water such as described in the WHO Guidelines for Drinking Water Quality and or that does not compromise food safety in the circumstances of its use* "(WHO/FAO, 2007). Whereas other national guidelines, for example, DIN 19650 (German standards) have strict limits and consider the water quality to be the same as drinking water containing no *E. coli* (Section 3.2.). The indicator microorganisms were above recommended limits in samples of surface water and of water used to fill the washing ponds (Table 3.5). However, the density of *E. coli* is probably underestimated due to the likelihood of high numbers of background bacteria or toxic substances that may interfere with the test and result in underestimation of the density of coliforms (APHA, 1999; Rompre et al., 2002).

This research highlighted the importance for testing the prevalence of enteropathogens in water used on farms and the need for risk assessment studies in the area of post-harvest washing to determine the contribution of water and contact surfaces to cross-contamination. In addition, it suggested further investigation of factors affecting the survival of enteropathogens and their infiltration inside leafy greens. Infiltration of wash-water into intact vegetables has been demonstrated with several fruits and vegetables, and is suggested to have been implicated in an outbreak of salmonellosis associated with fresh market tomatoes (FDA, 2015c).

Results in chapter 3 underpinned vigilant cleaning and disinfection practices at the consumers and retailers end. Bacteria that remain on the contaminated fresh vegetables after post-harvest washing will resume growing during handling such as distribution (Koseki & Isobe, 2005). For this, adequate knowledge in food safety and proper handling practices are essential for the safety of fresh produce. Chapter 4 showed that food safety knowledge of food handlers in the SMEs was generally inadequate. Despite that the trained group had a significantly higher mean score on knowledge (62.5±21.7) and self-reported practices (66.4±10.7) than untrained group, 52.2±19.6 and 57.6±14.3 respectively (Section 4.4.2.1 and 4.4.2.2), their awareness on issues related to temperature control and cross-contamination was in some cases inadequate. The results were in line with McIntyre et al. (2013) and Martins et al. (2012) who maintained that training alone is not sufficient if not backed up with continuous updates and learning process to enhance retention of information. Furthermore, the limited knowledge in basic food safety requirements raised concerns over the quality of trainings. In Beirut, training providers in food safety are few and their trainings are well recognized and advertised as CIEH or Highfield courses. These materials are not tailored to local needs or cultural practices in food operations, particularly to handling of fresh produce, including leafy greens.

The data also confirmed that the use of the KAP model to determine or establish an association to safe practices leads to misinterpretation of results. The combined approach of observation and interview surveys confirmed the disparity between self-reported and observed practices of food handlers (Figure 4.3) and presented further evidence on the limited application of KAP models when cultural diversity is not considered; but also, when knowledge in food safety is initially inadequate. In this context, the TPB maintains the greater the management support, motivating working environment, and resources, the more likely the food handlers' intentions are put into practice. It also denotes that individual intentions to embrace a particular behaviour will theoretically be enhanced with increased positive attitude toward their ability to perform a behaviour and positive feedback from important others (Ajzen, 2006). On the contrary, although the majority of respondents showed favourable agreement to food safety statements (86.3 ± 13.2 over 100 possible points), their attitudes were not consistent with self-reported practices (Section 4.4.3.1). Although the scores on attitudes and self-reported safe practices were generally greater for food handlers working in corporate-managed businesses than in sole proprietor-managed FSEs (Table 4.7), the association between attitudes and types of management was not strong (Figure 9.1; Section 4.4.3.2.). By this, results emphasized that food handlers' perceptions on management support or commitment can be influenced by their cultural background (relation and trust in business owner, understanding of hygiene, traditional practices), and by insufficient information on food safety requirements. This was confirmed by the low predictive values of management type in relation to self-reported compared to observed practices (Figure 9.1).

Along these lines, it showed that the understanding of the precise underlying factors for management commitment and management support are equally important, however beyond workers' perceptions. The undertaking of similar research at management leaders or decision makers level is particularly important in view of difference in cultural and food safety regulatory structure among countries, which are likely to impact consumers as well as food operators' behaviours and perceptions of food safety risks.

This issue was studied further in chapter 5 where two distinct types of management were compared in relation to food safety climates and control procedures.

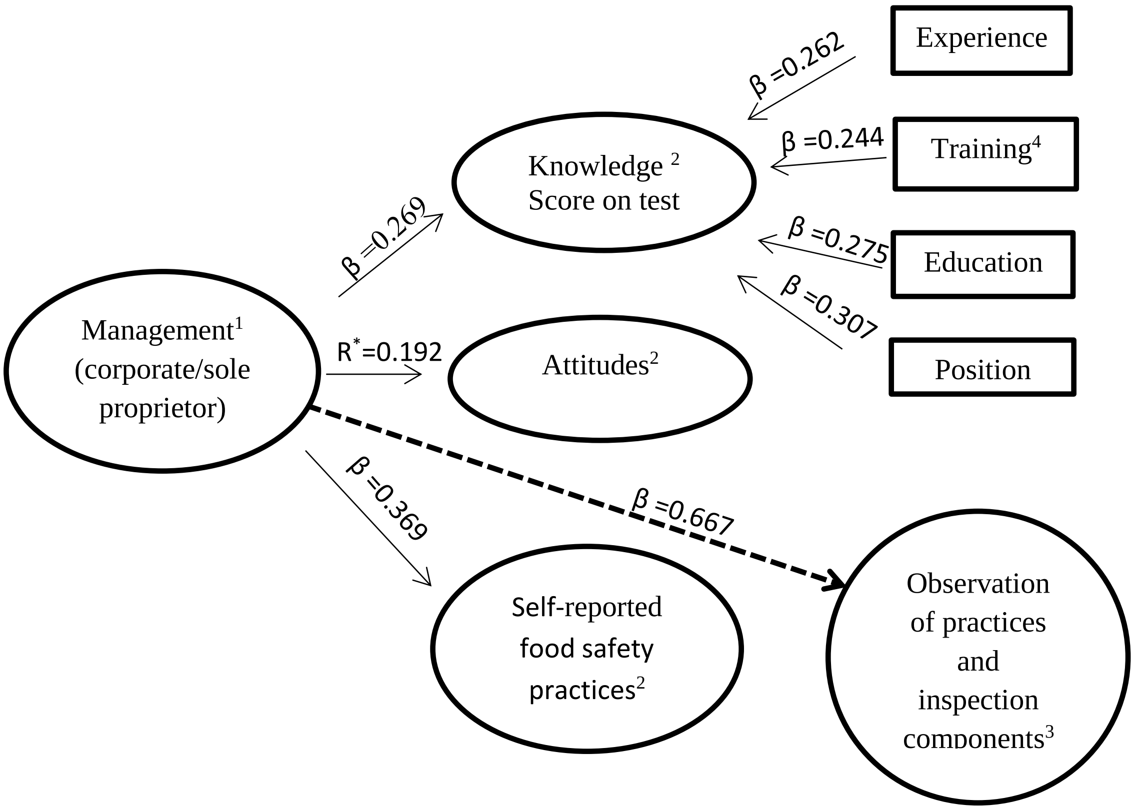


Figure 9.1. Regression analysis outputs depicting the predictive values of management and training as exploratory variables (independent variables) of safety practices and knowledge

[1]The predictive values of management types on KAP[2] and on observed practices[3]

[4] Predictive value of training on knowledge when other demographic variables were constant.

[*] Poor predictive role of management type on attitudes as confirmed by a weak association that approached significance (p = 0.056)

The corporate-managed businesses showed great emphasis on broader aspects of food safety, the pre-requisites that are technically familiar and easy to apply, than on integrating the key elements of a food safety system related to hazard-based preventive approach, time and temperature control, and internal control of food safety. The mean score of the overall visual assessment (77.88±18.45) was significantly higher than the recorded mean value of the sole proprietor businesses (48.47±12.82) (Section 5.4.1). In the context of fresh vegetables handling practices, the observation assessment revealed better washing practices of fresh vegetables in food operations managed by corporates (Section 5.3.). Nevertheless, temperature control, evidence of record keeping and documentation of internal control procedures for taking preventive measures were lacking and these are essential requirements for managing safe operations (FAO, 2010).

Therefore, advanced hygienic operations and superior personnel hygiene are likely to be much improved and maintained by supportive human resources capacity and a centralized management that executes food safety operations under structural framework, i.e., formal departments with assigned duties, delegations and span of managers control which encompassed the food safety department or operations department involved in ensuring the implementation of management decisions and hygiene requirements, contrary to operations restricted to few employees and to an executive role of owner or an executive chef in running day to day activities. Understaffing and limited management structure in small restaurants were identified as major constraints to safer practices (Fairman, 2004).

The firm size and the type of products (can be in this case branded services/products) are proven to be incentives that influence the motivation and perception of benefits for the adoption of food quality assurance systems (Seddon et al., 1993). A small firm handling an undifferentiated product will likely have a different perspective from a large firm handling a differentiated product. It is reported that the size of a company is also a driver for environmental performance and the main reason is the fact that large enterprises are more visible (Bran et al., 2010). In this respect, the corporate strategy is usually driven by stakeholders' trust and protection of corporate brands or reputation for adoption of sustainability issues (Manning, 2007). As noted in chapter 1 (Box 1.2), many small businesses operate in Lebanon without licenses shielded from the local authorities 'control, and these are often non-branded small Sole proprietor food outlets.

The present work raised additional research questions as to the main drivers for corporates with enabling environment and human capacity not to embrace a food safety system. It also highlighted other incentives that must be considered and measured in future research (Section 5.4.1). Reassurance of food safety on food premises basically entails that management ensure availability of food safety standards (Clarke, 2000). Taking into consideration the financial constraints as an additional factor in the case of sole proprietor groups, the comparative analysis denoted the relevance of addressing the food safety values of management or owners of food businesses in future research and that food operations should progress beyond the fundamental prerequisites and supportive environment towards embedding those values and ethics in their policy and by viewing food safety as a critical issue.

Considering the variations in cultural background and in the food safety framework among countries, the results bring into attention the complex interplay of ethical practices and values as associated with the perceptions of food safety risks. Consumers as well as food chain stakeholders' awareness on food safety aspects and risks associated with practices are affected by local authorities' interventions in food safety and risks communications. Several studies pointed at food safety laws and bylaws, as well as consumer demand, to be the key external incentives for adoption of food safety assurance standards (Henson and Caswell, 1999) and their influence varies with different cultural and regulatory systems. According to Korthals (2004), the perception of risks arises as a function of trust in the authority that defines and sets out the policies for risk management and adopt transparent risks communications (Korthals, 2004). Although little is known on organization's perceptions of risks or attributes that induce ethical practices in relation to food safety, this is a topic that is being researched to understand consumers' purchasing behavior and attitudes towards new practices (Redmond & Griffith, 2004). For example, consumers' attitudes towards emerging food technologies was largely determined by their ethical consideration and values i.e., their concerns about the integrity of nature (Frewer et al., 2007), which reflects in turn the importance of understanding the food safety values of the management's leaders/decision makers. Korthals (2004) pointed at the food safety issues to be closely related with other socio-ethical issues in the food chain, such as sustainability, animal welfare, human health, and respect for small farmers in developing countries

Although food safety decisions and food safety policy are based on "science", they are also affected by economic considerations and benefits (FAO, 2003). There are five groups of values inherent in decisions about food safety policy: the right to adequate food, trust, optimization, informed consent and equity. "The right to adequate food is fundamental to food safety policy considerations because it responds to the universal human right to safe and nutritious food, and because it encompasses other human rights such as the right to information, culture and human dignity" (FAO, 2003). In this context, values and ethical dimensions may also play a central role in management decisions for the adoption of food safety reassurance system and instilling a food safety culture. Unless an organization or enterprise is consumer-oriented and food safety is equally addressed into corporate beliefs and values as profits concerns, compliance of food handlers may not be adequately resolved.

The inadequate handling and contamination levels at the post-harvest washing stage underscore the importance of food operators and food handlers' attentiveness in handing RTE fresh vegetables. The deficiency in food safety knowledge, together with the lack of food operators' commitment or awareness to apply preventive measures, had some bearing on the handling practices and production environment. Effective sanitization and cleaning procedures represent critical points that are essential to reduce the likelihood of bacterial hazards and to improve hygiene and these were found lacking in the SMEs and in some cases ineffective. Chapter 6 showed critical deficits in cross-contamination prevention measures that included lack of sanitization and cleaning standard operating procedures on food premises, besides improper storage conditions in SMEs with a limited working space. Consequently, the microbiological quality of RTE vegetables, originally purchased from the wholesale market, was found to be unsatisfactory in more than half of the RTE salad vegetables (Section 6.4.2.and 6.4.3.). *E. coli* and *Listeria* spp. counts exceeded the criteria limits >10^2 CFU/g and *S. aureus* was isolated from 41.5% of the samples. This work confirmed that a poorly rated restaurant does not necessarily means poor microbial quality of fresh vegetables sampled from that same outlet (Section 6.5.3.) and that the total score on visual assessment is not a reliable tool to judge the safety of fresh vegetables. Therefore, end-product testing of salads vegetables can't provide safety. It is established that using microbiological analyses of surfaces and applications of critical control points based on factors that were found most likely to be responsible for high microbial levels on RTE vegetables is an essential method to reduce microbial hazards.

Results in chapter 7 showed that none of the tested washing solutions used in the SMEs effectively eliminated *S*. Typhimurium on parsley. This chapter presented new information on the critical role of temperature control during handling fresh parsley in SMEs, chiefly in countries with warm climates, for the effective elimination of pathogens on intact and fresh -cut parsley. Interestingly, it was found that the improper storage conditions (30°C) altered the efficacy of all tested washing methods; however, the decontamination effect was optimized in the cold condition (5°C). The highest log reduction was observed on unchopped parsley at low temperature (5ºC) (Section 7.4.2.), while the decontamination effect of all solutions was the least effective at higher temperature (30°C) on chopped leaves. Additionally, this research proved that NaDCC is an effective economic sanitizer to be used in SMEs with parsley and probably other leafy greens as it showed the highest decontamination effect ranging from -1.92 to -3.12 log reduction on unchopped parsley. The benefits of

NaDCC lie in its prolonged effectiveness with the advantage of leaving no odour or taste (Clasen & Edmondson, 2006), besides that chemical compounds based sanitizers such as the inorganic chlorine compounds have been reported to produce hazardous by-products (FDA 1998; Kim et al. 2012) and adverse effect on the sensory quality (Beuchat and Ryu 1997).

The common cleaning procedures of CB did not to eliminate the cross-contamination risks during the chopping process. Chapter 8 demonstrated that in such conditions of poor hygiene and inadequately washed and non-sanitized cutting boards after use with a contaminated parsley, remaining *Salmonella* cells on CB were capable of survival for 24 h at 30°C on washed CBs and were transmitted subsequently chopped batches of fresh clean parsley. As hypothesized, the pathogen transmission continued across all batches, however a significant drop in Tr level was only observed at the early stage of chopping (Section 8.4.2.1). This research showed that the Tr of *S.* Typhimurium to parsley was strongly and negatively correlated with the contamination levels at source (Section 8.4.1). Low contamination levels of *Salmonella* on parsley leaves can therefore still contaminate a number of clean batches of parsley with a high Tr (60%-64%) and still present a potential health risk to consumers. The sanitization of CBs used for fresh produce is paramount, particularly when food safety measures against bacterial contamination at sources is not assured in Lebanon.

9.2. Conclusion

In Lebanon, the challenges in food safety are complex. The socio-economic and political factors can affect the implementation of changes and safe practices along the food value chain. Nevertheless, the primary reasons clearly surpass the socio-economical drivers and are rather attributed to the absence of a national food safety policy that addresses local consumers' health through robust government regulations and implementation of measures to assure the safety of vegetables in the local market.

It is proposed that food safety interventions in Lebanon should focus more on the protection of local consumers' health by increasing consumers and stakeholders' awareness on food safety risks and by directing incentives and agricultural funds towards the local fresh produce production - even though strict local regulations are still absent. Increasing consumers' awareness on existing food safety risks and about the quality of their food supply in Lebanon. Consumers' more aware decisions and purchasing behaviours offer new opportunities to impact positively on the overall food safety governance system and on food operators or producers' adoption of safer practice through the (CIS, 2004). Western consumers are an example on their increased interest in the quality and safety of food, sources and processes that they pressure government to take greater responsibility for food safety and consumers' health protection (FAO/WHO, 2003).

For this, the objective should be to draw producers' attention more intensely on microbiological hazards and the introduction of food safety management. HACCP needs to be built on the local data, local agriculture environment, processes and skills, which was served in this study, rather than directly transferring models from different countries. Overall, this research emphasized on effective control measures to reduce contamination at the source. This should include runoff control structures, the protection of surface waters and private wells from uncontrolled livestock access to limit the extent of fecal contamination.

The trend described here, could also occur in other countries of the MENA region in view of common socio-economic and cultural features and the limited applications of GAP. Although, the number of producers included in this study should not be used to infer to the general population, the data still bear a considerable level of reliability and scientific significance. The sample presented producers in an extensive agricultural area of Lebanon, where the quality of water resources, infrastructure, agricultural and rural challenges are common for many other farms.

At the lower end of the chain, the research emphasized that theoretical models to understand determinants of food handlers' practices should be complemented with observational data as food handlers' misperceptions can greatly affect the interpretation of safe practices and attitudes associated with other determinants. As management structure was a determinant of food handlers' behaviour and of improved environment, the understanding of factors that hamper or enable the management is instrumental in developing food safety interventions. It is suggested to evaluate the food safety values within the social context at management level to form a reasonable judgment on impediments for a food safety culture and an opinion on the local food safety issues in order to develop tailored solutions. Although the corporates could compensate the relative absence of public sector role, risky behaviours were still recorded in these establishments. Hence, the risk of foodborne disease transmission via food workers can be effectively reduced with a progress towards a comprehensive system to reassure food safety. This requires a recognition of the food safety risks, and most importantly that they embed food safety in their core values in order to foster safer practices (Figure 9.2). The Figure 9.2 presents inputs generated from the primary data collected through interviews, visual assessment and knowledge.

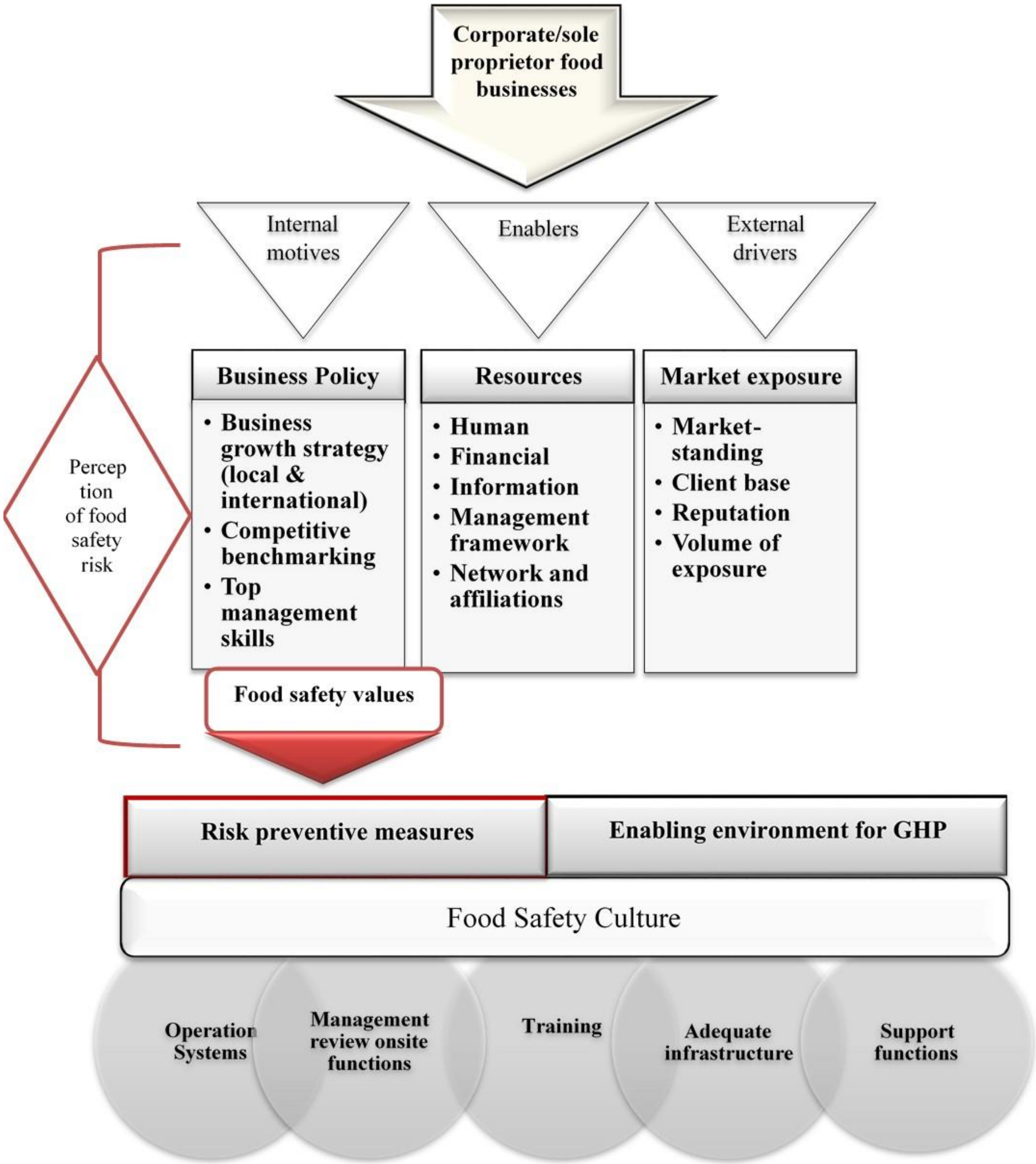


Figure 9.2. The food safety values and ethical dimensions essential for food safety culture

The outcomes of this research underlined the need to foster ongoing educational support and technical guidance at all levels as a priority to reinforce the food safety values and integrate them into practices in SMEs. Even though the sample size should not be used to generalize the findings, there are common cultural characteristics well known in a country like Lebanon that allow a certain degree of certainty that the present data reflect common features of FSEs in the sector at large. However, further research in this area will be needed.

A cluster of governmental support and regulations should provide the food service sector with guidelines and educational programs. Development of food safety systems that can be rationalized without additional burdens such as the “Safer food, Better business” by the Food Safety Agency in UK is another example for the local authorities to build on. This system offers a practical and simple documentation system, for the SMEs “Diary” which is essential for the food safety assurance and for the implementation of critical control points to improve the food safety of fresh produce particularly when prepared in small working facilities.

Drawing from information generated in this research, a conceptual framework model was developed to assist in determining policy responses and framing various factors. The model depicts drivers that impacted practices based on the combined primary and secondary data, which in turn had direct effect on the microbiological quality of fresh vegetables (Figure 9.3). The effective strategy that could be drawn up at this stage to minimize the risk of microbial contamination at all points from the field to the table should be through:

i) Developing national guidelines for the appropriate implementation of food safety management systems, including GAP, GHP and GMP, as well as incentive schemes to adopt GAP standards. Such incentive schemes need to be tailored to farmers’ capabilities., e.g., introducing a modular certification system which allows a gradual adoption of the standards while considering critical points for compliance based on the local environment. The guidelines should address the microbial hazards and safe practices during growing, harvesting, washing, and transportation; however, current concerns in Lebanon are focused on chemical hazards and the legal framework does not specify microbiological criteria applicable at primary production of fresh produce to validate and verify GAP, GHP or HACCP. *E. coli* was defined as hygiene criterion for this purpose by EFSA (2014), *Salmonella* which was isolated from lettuce on farms and retails should also be proposed and communicated to producers and processors as a food safety criterion in leafy greens that, according to EFSA (2014), should not be present in the produce.

ii) Further investigations on the prevalence *S. Aureus* on produce and its association to sewage and chicken manure on fields should be taken to assess its potential significance as an indicator on farming practices.

iii) Implementation decree for the ratified food safety law which encompasses accountability and requires improvements in the quality infrastructure of the fresh produce chain.

iv) Enforcement of water policy, improvement in water infrastructure and monitoring program of water quality used for irrigation and post-harvest washing.

iv) Setting requirements, clear guidelines for the implementation of prevention measures of cross-contamination, and for adequate storage and temperature conditions during the washing, storage and transportation stages. Improved transportation requires that cold chain transportation should be enforced. Washing of fresh produce in trucks should be abolished and where possible, refrigerated vehicles should be introduced.

v) Developing and applying strict policies on workers' health and hygiene, safe sources of water and on post-harvest washing processes.

vi) Strengthening the capacities of food controllers and inspectors and provide them with the needed equipment.

vii) Applications of mandatory programs for food safety education and capacity development of food chain operators on fresh produce handling in accordance to food safety standards.

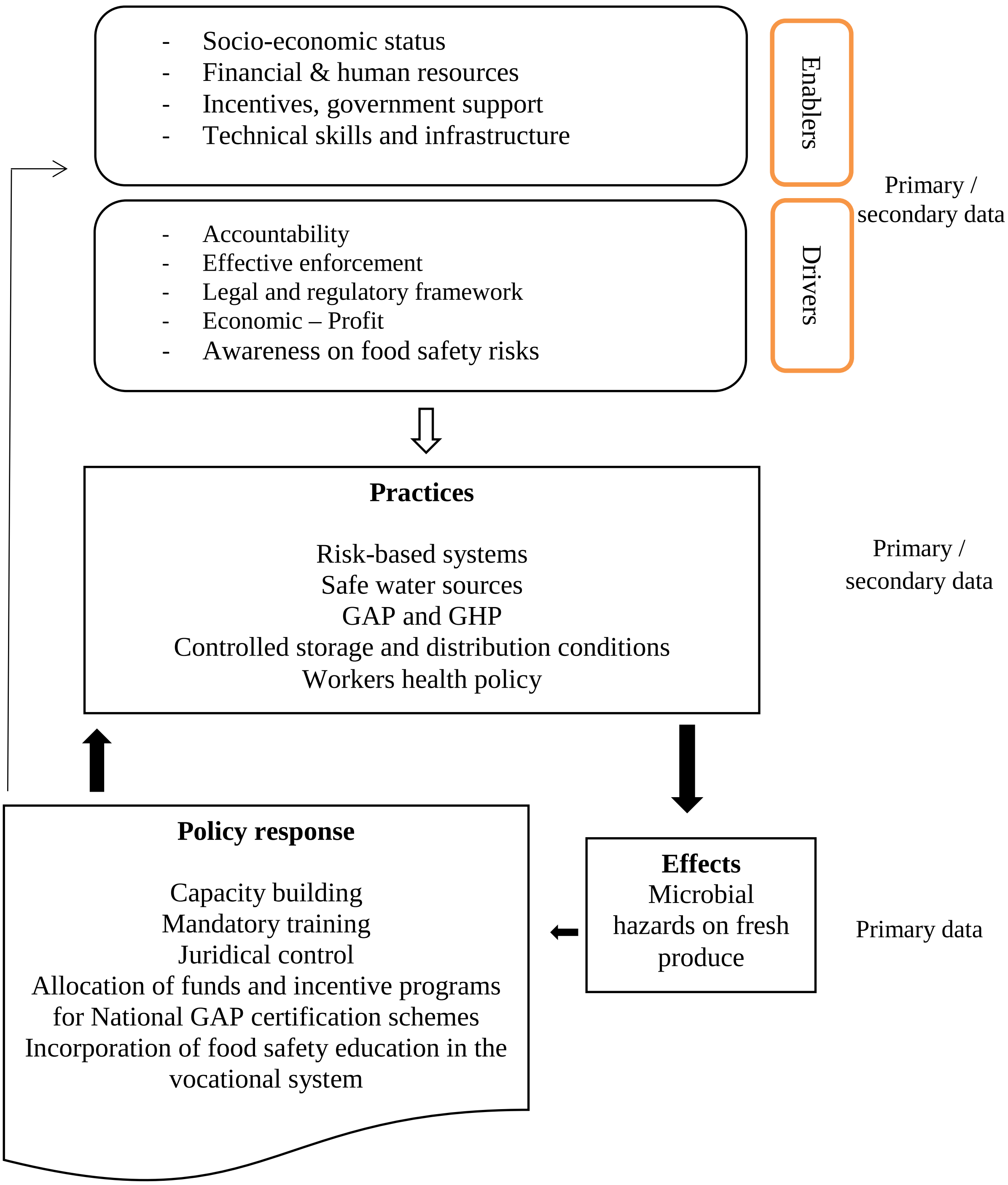


Figure 9.3. Conceptual framework of different parameters related to safety of fresh produce in primary production environment

9.3. Future work

Additional experiments were undertaken as part of the farm-to-fork study concept. There is ongoing work on data related to studies on,

1- The antibacterial effect of traditional pomegranate sauce on chopped parsley. The antimicrobial properties of pomegranate molasses(PG) and its mixture with salad sauce was tested against *S.* Typhimurium on parsley leaves in a traditional salad.

2- The efficacy of washing methods that were studied in chapter 7 on reducing the contamination levels and attachment of *L. monocytogenes* on parsley under different temperature conditions and relative humidity.

3- Determination of the Tr of *L. monocytogeness* from contaminated wash water to clean parsley.

9.4. Limitations

The research limitations were mainly related to a limited funding; hence a larger sample size of fresh produce was not possible. In addition, the deteriorating security conditions in the rural areas was a significant limitation that affected the course of the field work. Overall, the field work on farms was scheduled in the summer, which might be biased in this case as it captured snapshots observations and sampling at one point of the year. Future studies that focus on the seasonal variations could build on the existing work and deliver comprehensive information on the microbial hazards on fresh produce in the given conditions. Nonetheless, this research provided good baseline data on common gaps in hygiene practices along the fresh produce chain. In addition, norovirus was not investigated, which undoubtedly was present from any human sewage sources, and would present a further health risk to consumers.

Due to logistical limitations, analysing the food samples collected from farms to market within 24 h of collection was not possible, and these were frozen and thawed before analysis.

From this, it is assumed that the freezing and thawing likely led to some decline in the reported bacterial counts, which could have been higher than what is actually documented.

The sample size in the current study did not include farms from different areas in Lebanon; thus, the generalization could not be inferred to all types of farms and further investigations are needed; nonetheless, this thesis has contributed to literature and provided parameters for other studies with statistically valid sample size to ensure the validity of their research results.

APPENDICES

APPENDIX A

REGULATIONS OF FOOD SAFETY IN THE MOPH – EMAIL COMMUNICATIONS

Below is a copy on a series of email correspondences that addressed an inquiry from the regional FDA office. This copy was received during the personal communication phase of the thesis with the head of departments in the MoPH in request for additional information on the food safety system in Lebanon.

Dear Dr Ammar
This is what I got from Mrs Samis Chatila who was dealing with food safety at the CPHL (Answers are in red). I am also waiting for Mrs Rendala Noureddine's opinion since she has also been working on this issue.
Sincerely

Atika Berry MD, MpH
Head of The Communicable Diseases Dpt
MOPH, Lebanon
Tel: 00961 1 611844-5
Fax: 00961 1 615720

From: mphealth@cyberia.net.lb
To: aberrymd@hotmail.com
Date: Tue, 12 Feb 2013 07:21:35 +0200
Subject: Fwd: Questions related to Food Safety

--Forwarded Message Attachment--
Date: Mon, 11 Feb 2013 16:01:28 +0200
From: ChammaMS@state.gov
Subject: Questions related to Food Safety
To: mphealth@cyberia.net.lb
CC: rashahamra@yahoo.com

Dear Dr. Ammar,

I hope this finds you well. Following an inquiry we received from the regional FDA office, and in order to facilitate trade between Lebanon and the U.S., I would appreciate your kind assistance in answering the following questions on food safety.

1. Which aspects of food safety does the Ministry of Public Health (MoPH) regulate?

Microbiology aspects are controlled by refering samples for culturing to FANAR LAB at Ministry of agriculure.

2. What are the major laws under which regulations for food safety are issued?

LIBNOR institute is the authority at Ministry of Industry that issue official NORMS for different food items and other matrix.

3. What are the major regulations controlling food safety such as food GMPs, inspection, color additives regulations, nutritional supplements, labeling, etc.?

LIBNOR is an ISO member,having mirror committees to follow updated regulations in original norms, and hence most our norms are NL ISO for different food subjects ,we also follow Codex Alimentarius and GMP.

4. Who controls food exported from Lebanon?

custom Inspectors ,Inspectors of department of consumer protection, inspectors of agricultre.....

5. Does the MoPH require proof that exported food meet the requirements of the imported country such as the USA?

Certificate of analysis of the producing country is requested usually.

6. Does Lebanon have its own microbiological standards or does it follow international organizations?

The Lebanese Norm includes the microbiological limits of each product ,however if not, we might refer to international guidelines (WHO,codex alimentarius,EPA....)

7. Does Lebanon have a food recall system?

yes ,The ministry of economy and trade have deffined bodies to recall non complying items or the police may do the job of searching suspected stores.

Should you need any further information, please do not hesitate to let me know. Thanking you in advance for your cooperation in this matter.

All the best,

Marina
Marina Chamma
Economic Specialist
U.S. Embassy – Beirut
Tel.: +961-4-542600/543600 Ext.: 4487
Cel.: +961-3-240654/ Fax: +961-4-544794
Email: chammams@state.gov
This email is UNCLASSIFIED

QUESTIONS RELATED TO FOOD SAFETY

1.Which aspects of food safety does the Ministry of Public Health (MoPH) regulate?

1. Food Supplements. (see Regulations below)
2. Infant milk formula (MPH 212-15- requirements)
3. Bottled Water and beverages (Decree- 108-1983)

2. What are the major laws under which regulations for food safety are issued?

1. Legislative Decree No. 108 for the year 1983 on the regulation of investment in canned carbonated water and beverages
2. Legislative Decree No. 71 for the year 1973, safety of all classes of food (articles inconsistent with the new consumer protection law shall be cancelled);
3. Legislative Decree No. 73 for the year 1983, as amended by Decree No. 72 for the year 1991, on possession and trading of commodities, materials and crops (articles inconsistent with the new consumer protection law shall be cancelled);
4. Legislative Decree No. 4880 for the year 1966 on imposing legal obligations regarding standards and specifications for certain foodstuffs;
5. Legislative Decree No. 12253 for the year 1969 on the condition of canned and preserved food products;
6. Law No.13068- 2004-Consumer protection Law
7. Decree No. 5243 for the year 2011 on the classification of Manufacturing Companies articles related to Food Manufacturing (ICIC No. D 15 (1511-1598)
8. Decree No. 5765- 2003 on Inspection on Manufacturing Companies related to public Health safety including manufacturing, production of food commodities.
9. Decision No.105-2002 (Ministry of Agriculture- Meat and Meat products Plant registered.
10. Decision # 272/1 dated 14 Mar. 2011
11. Decision No.822-2010 (Ministry of Agriculture- Milk and Milk products registration)
12. Decision No.821-2010 (Ministry of Agriculture- Milk and Milk products Transportation and cold chain.
13. Decision No.1043-2011 (Ministry of Agriculture- Milk and Milk products packing and trading)

3. **What are the major regulations controlling food safety such as food GMPs, inspection, color additives regulations, nutritional supplements, labeling, etc.?**

- LIBNOR Standards : 656 related to GMP
- LIBNOR Standards: 605 related to HACCP
- LIBNOR Standards195: Salmonella levels in Poultry Products
- LIBNOR Standards: 654 related to manufacturing and handling frozen food commodities and products.
- Decree No.11710 dated 22 January 1998 (MOPH)

- Law No. 530 dated 21 July 2003 (article-4) (MOPH)
- Law No. 90 dated 6 Mar. 2010 (MOPH)
- Decision No. 844/1 dated 1 Sept. 2010 (MOPH)
- Decree No. 5518 dated 14 Dec. 2010 (MOPH)

    1. Diet Products
    2. Vitamins & Minerals
    3. Concentrated Foods
    4. Sports Nutrition
    5. Uppers other than Pharmaceuticals & Energy Drinks
    6. Natural Plants & Herbs with medicinal benefit
    7. Covers Import, Manufacturing, Packaging & Packing

4. **Who controls food exported from Lebanon?**

- Ministry of Industry
- Ministry of Agriculture
- Ministry of Economy and Trade

5. **Does the MoPH require proof that exported food meets the requirements of the imported country such as the USA?**

No

6. Does Lebanon have its own microbiological standards or does it follow international organizations?

Most food commodities are required to follow the Lebanese Standards Requirements updated by LIBNOR (Lebanese Standard Institution).
In Cases where Lebanese Standards are not present, International Codex standards will be the reference.

7. Does Lebanon have a food recall system?

- Yes, the food recall system is under the custody of the Ministry of Industry in cases of canned and processed and imported food products.
- Ministry of Health is responsible for food recall of any Infants Formulas and Food Supplements
- Ministry of Agriculture is responsible for food recall of fresh milk and plant products.

NB: LIBNOR is an ISO member, having mirror committees to follow updated regulations in original norms, and hence most of the Lebanese norms are NL ISO for different food subjects, they also follow Codex Alimentarius and GMP.

APPENDIX B

SAMPLE CRITERIA FOR RESTAURANTS' ASSESSMENT

معايير التصنيف العائدة للمطاعم

رقم	المعايير	★	★★	★★★	★★★★	★★★★★	الملاحظات
	عموميّات						
٦	إن المطعم الحا ئز على شهادة HACCP "نظام تحليل المخاطر وتحديد النقاط الحرجة" أو ISO ٢٢٠٠٠ يعتبر ذو درجة ممتازة في فئة تصنيفه و يصنف بحسب نجوم تصنيفه مع زيادة "S"						تعني "ممتاز" كلّ المطاعم الحائزة على شهادة "نظام تحليل المخاطر ومعالجة النقاط الحرجة "HACCP" أو شهادة ISO ٢٢٠٠٠. هذه الدرجة مستقلة عن فئة التصنيف وعدد النجوم (تتعلّق هذه الشهادات بالطعام والمشروبات)
٧	الشروط الصحية والنظافة والصيانة الدورية هي من أساسيات التصنيف.	X	X	X	X	X	خارج وداخل المؤسسة (بما في ذلك مكان استلام المأكولات، الممرات والمداخل من المطبخ منظمة جيداً ونظيفة). الأماكن العامة "الأسقفة والأرضيات في حالة جيدة ولا تظهر علامات القدم والاستعمال. المباني والتجهيزات مصا نة بشكل جيد وهي بحالة نظيفة. الطاولات والمقاعد في حالة جيدة ونظيفة. ، الخ...
٨	تأمين مورد كهربائي ثاني (مثل مولّد كهربائي أوغيره) من أجل توليد الكهرباء في المؤسّسة لتشغيل جميع التجهيزات الأساسية على مدار الساعة	X تجهيزات اساسية باستثناء جهاز التكييف	X تجهيزات اساسية باستثناء جهاز التكييف	X	X	X	
٩	إنارة جيدة في الأماكن العامة وكافة أقسام المطعم.	X	X	X	X	X	
	الوقاية من الحريق						

معايير تصنيف المطاعم - إصدار ٢ ٣/١١

SF/……./…

APPENDIX C

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

CONTACT INFORMATION

A- Farm information

Farm Area Location: ____

B- GAP and GHP training:

Did you participate in GAP[1] and GHP[2]: YES ☐ NO ☐

If yes, describe

____

____

____

[1] Good Agricultural Practices
[2] Good Hygienic Practices

SF/……./…

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

SECTION I- GENERAL INFORMATION

	Production volume	Market/Region supplied to
Parsley		
Mint		
Lettuce		
Bakleh		
Cucumber		
Radish		

SECTION II- WATER USAGE AND SEWAGE TREATMENT

1. **Describe the MAIN source of water used for irrigation and application methods:**

____

____

____

____

2. **How do you tell if the water you are using for irrigation doesn't carry any potential pollutants from nearby livestock, wildlife, and other potential sources?**

____

3. **Is there any municipal/commercial sewage treatment facility or waste material landfill adjacent to the farm?**

YES☐ NO☐

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

If yes, describe how near is it to the farm:____

4. **Is the farm sewage treatment system/septic system functioning properly and there is no evidence of leaking or runoff.**

YES☐ NO☐ N/A☐

If yes, how often is it maintained and monitored?__________________________________

SF/……./…

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

SECTION III- ANIMALS/WILDLIFE LIVESTOCK

---

1. **Controls are in place to decrease contamination of agricultural water and soil from other farm or animal operations.**

   Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

   When applicable, describe the existing control system:________________________________

   ____

2. **Manure lagoons located near or adjacent to production areas are maintained to prevent leaking or overflowing, or measures have been taken to stop runoff from contaminating the production areas.**

   YES ☐ NO ☐

3. **How often are domestic and wild animals kept away from water used for irrigation and the production area?**

   Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

SECTION IV- MANURE AND MUNICIPAL BIOSOLIDS

---

1. This farming operation applies:

A. Raw manure
B. Combination of raw and composted manure
C. Composted manure
D. Other

2. If this farming operation applies raw manure or a combination of raw and composted manure as a soil amendment it is incorporated immediately at least 2 weeks prior to planting or a minimum of 120 days prior to harvesting.

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

3. **If a combination of raw and treated manure is used, how is it treated to reduce the expected levels of pathogens**?

____

____

____

4. Is the manure stored prior to use?

YES ☐ NO ☐ N/A ☐

If yes, how is it stored?________________________________

____

5. Are composted manure and/or biosolids treated to minimize recontamination?

YES ☐ NO ☐ N/A ☐

If yes, how are they stored?________________________________

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

SECTION V- SOILS

If flooding occurred in the crop production areas, are soils tested for potential microbial hazards?

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

SECTION VI- TRACEABILITY

Each field is coded or identified to enable traceability in case of a recall

YES ☐ NO ☐

SECTION VII- FIELD HARVESTING, STORAGE AND TRANSPORTATION

1. Time/Temperature history

	Time of Harvest	Storage duration	Storage temperature condition	Transportation time
Parsley				
Mint				
Lettuce				
Bakleh				
Cucumber				
Radish				

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

2. Are storage facilities available?

YES ☐ NO☐

If yes, the produce are stored in :(possibility to choose more than one option)

A. Containers
B. Bags
C. Closed , sealed, ventilated facility
D. Open facility
E. Packed on racks
F. Other______________________

3. Is any produce co-mingled from different producers in storage?

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

4. All harvesting containers as well as hand harvesting implements that come in direct contact with harvested produce are cleaned and/or sanitized prior to use and kept clean

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

Comments:____

5. All harvesting containers will be used solely for the carrying or storage of the intended crop?

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

6. Transportation equipment used to move produce from field to storage areas or storage areas to the markets which comes into contact with product is clean, in good repair and covered.

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

7. Product moving out of the field is coded or identifiable to enable traceability.

SF/……./…

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

YES ☐ NO☐

8. How often is the storage facility cleaned?

Always ☐ Often ☐ Sometimes ☐ Seldom ☐ Never ☐

9. Are storage rooms, buildings and/or facilities sufficiently sealed to protect from external contamination?

YES ☐ NO☐

10. How do you rate the maintenance level of the storage facilities and floors?

Excellent ☐ Above average ☐ Average ☐ below Average ☐ poor ☐

11. How do you think of the cleanliness level of the mechanical equipment, pallets and boxes used within the storage facility?

Excellent ☐ Above average ☐ Average ☐ below Average ☐ poor ☐

12. Mode of transportation and packaging materials utilized

A. Open Lorry
B. Closed Lorry

13. Packaging material during transport

A. Baskets
B. Sack bag
C. Plastic crates
D. Other________________

SF/……./…

SURVEY ON FARMS

AGRICULTURAL & HYGIENIC HANDLING PRACTICES

SECTION VIII- WORKER HEALTH AND HYGIENE

Worker Health and Hygiene	
All employees have been trained and are required to follow proper sanitation and hygiene practices. Employee name, date of training, and training method are documented	Yes ☐ No ☐
Readily understandable signs are posted in appropriate areas to instruct employees and visitors to wash their hands before beginning or returning to work or when their hands have been contaminated.	Yes ☐ No ☐
All toilet/restroom/field sanitation are serviced and cleaned on a scheduled basis. They are properly supplied with single use paper towel, toilet paper, and hand soap or anti-bacterial soap and potable water for hand washing.	Yes ☐ No ☐
Eating, drinking, chewing gum and tobacco use are confined to designated areas separate from where produce is handled. Designated areas include Bottled water is allowed provided it is stored in closed plastic containers away from the product flow when not being used.	Yes ☐ No ☐
Workers with flu-like symptoms or open wounds, or infectious conditions are prohibited from handling produce.	Yes ☐ No ☐
A written policy is in place whereby the harvest that have come in contact with blood or other body fluids will be disposed using the most appropriate method for the situation (e.g. buried, burned, etc.). Equipment surfaces that have come into contact with blood or other body fluids will be cleaned and disinfected with bleach or other safe disinfectant.	Yes ☐ No ☐
First aid kits are identified, checked and restocked on a regular basis. All employees are instructed to seek prompt treatment with clean first aid supplies for cuts, abrasions, and other injuries. Workers are instructed to report any injuries to their supervisors and will be documented in the illness/injury reporting log.	Yes ☐ No ☐
Any pesticide, fertilizer, or nutrient applied in the production of the crop will be documented and kept on file. Company personnel applying regulated materials must have name and pesticide license recorded and on file.	Yes ☐ No ☐

APPENDIX D FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

A. General Information

1. Food Service Establishment:

Catering ☐ Restaurant ☐

2. Location/Area:___________________________________

B. Demographic characteristics

Age:________ Gender: M☐ F☐ Length of service:________

Experience in food service:_________________

Position:________________________________

Education level:__________________________

Attended courses on food hygiene/safety: Yes ☐ No ☐

If Yes, state the courses attended:____

____

FAP/.......

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

C. Food handlers knowledge

1. When fruit and vegetables are delivered, we should

A. check the hygiene condition of delivery vehicle
B. check the temperature of fruit and crops
C. check whether the driver is respecting personal hygiene principles
D. check the quality of fruit and crops
E. I do not know

2. The correct temperature for a refrigerator is:

A. <1°C
B. 1-5°C
C. 6-10°C
D. 11-15°C
E. I don't know

3. The temperature in your freezer should be:
A. -2°C
B. -9°C
C. -12°C
D. -18°C
E. I don't know

4. Hot ready to eat food should be maintained at:

A. 21-30°C
B. 31-40°C
C. 41-50°C
D. 51-60°C
E. 61-70°C

5. What is the appropriate temperature for keeping a salad dish containing chicken?

____

6. Which of the following are most likely to cause bacterial contamination?

A. Food handlers
B. Insects
C. Raw materials
D. All of the above
E. I don't know

FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

7. In order to prevent foodborne illness, left overs should be reheated to?

A. 60°C
B. 65°C
C. 74°C
D. 80°C
E. I don't know

8. Do you know what is the danger zone (temperature range) when bacteria in food are more likely to multiply?

Yes ☐ No☐

If Yes, explain :____________________

9. Could you tell if the food is contaminated with bacteria causing foodborne illness?

Yes ☐ No ☐

10. If yes, how do you tell that the food is contaminated with bacteria causing foodborne illness?

A. Taste changes
B. Appearance
C. Smell
D. Color
E. I do not know

11. Which one of these foods is likely to contain the most bacteria?

A. Cooked chicken
B. Tinned coconut milk
C. Frozen raw chicken
D. Bottled Mayonnaise
E. All of the above

12. Which condition kills bacteria?

A. Cooking
B. Cooling
C. Freezing
D. All of the above
E. I don't know

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

13. In refrigerators temperature, the bacteria

A. Multiply
B. die
C. grow very slowly
D. do not grow
E. I do not know

14. Which is a common symptom of food poisoning?

A. Headache
B. Diarrhoea
C. Rash
D. Constipation
E. I do not know

15. What should a food handler do when he/she has diarrhoea ?

A. Take his medicine and continue working
B. Inform the supervisor and continue working
C. Avoid handling foods 48 hrs. after symptoms alleviation
D. I do not know

16. When the room temperature is 26 °C or above, cooked food should not be left-out longer than

A. One hour
B. 2 hours
C. 3 hours
D. 4 hours
E. I don't know

17. When should you wash your hands?

A. After handling raw meat
B. After handling raw eggs
C. Before handling cooked and RTE foods
D. All of the above
E. I don't know

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

18. Where should we place the thawing meat in the chiller?

A. On the uppermost rack
B. In the lowest rack
C. In the middle
D. It doesn't matter
E. I don't know

19. Where in the cooling unit that contains fresh meats would you store the prepared salads?

A. On the highest rack in the refrigerator
B. Next to the meat
C. On the rack under the meat
D. Other_________
E. I do not know

20. After using the knife for cutting raw meat, it should be

A. wiped with a kitchen cloth
B. thoroughly washed and disinfected
C. thoroughly washed with boiling water
D. thoroughly washed under running water
E. wiped with a paper towel
F. I do not know

21. Should raw and cooked foods be separated?

Yes ☐ No☐ I don't know☐

Explain why?______________________

FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

D. Food Handlers Practices

D.1- Please answer by ticking "X" on the corresponding answer: Always, Often, sometimes(ST), Seldom or never.

	Always	Often	ST	Seldom	Never
Are fresh vegetables delivered from one supplier/source?					
Are fresh leafy vegetables or/and pre-cut vegetables delivered cooled?					
Do you wash the vegetables in a separate designated sink?					
Is the washing water used for fresh vegetables and fruits chlorinated?					
Do you measure the temperature of received frozen and fresh meat products?					
Do you take measurements of the cooler and freezer on your premises?					
Do you measure the temperature of food during cooking?					
Do you measure the temperature of food during reheating and cooling?					
Do you disinfect cutting boards and utensils for raw meats?					
Do you use separate cutting boards and utensils for raw meats ?					
Do you use gloves when you touch or distribute unwrapped foods?					
Do you wash your hands before using gloves?					
Do you wash your hands after using gloves?					
Do you use protective clothing when you touch or distribute unwrapped foods?					
Do you use a mask when you touch or distribute unwrapped foods?					
Do you wear a cap when you touch or distribute unwrapped foods?					
Do you check the expiry date on delivered product?					

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

Do you wash the vegetables before cutting?					
If applicable: how often you record the temperature of the display salad bar?					
The received fresh vegetables are kept in the cold storage room/fridge					
The washed and cut vegetables for salads and garnishes are held at room temperature before preparation/service					

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

D.2 Please state how you wash the fresh vegetables i.e. tomatoes, cucumber, and radish? *choose more than one answer if applicable on your premises

A. Soaking then washing with cold water
B. Soaking then washing with warm water
C. Washing with a brush
D. Sanitizing ________________
E. Other____________________

D.3 Please state how you wash the fresh leafy vegetables i.e. lettuce, parsley, mint? *choose more than one answer if applicable on your premises

A. Soaking then washing with cold water
B. Soaking then washing with warm water
C. Sanitizing with________________
D. Other_________________

D.4 Please State the time and temperature conditions of storage, preparation and display of the salad vegetables

Produce/Salad mix	Storage time/temp.	Preparation time/temp.	Holding time/temp.

FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

Comments:

FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

Food handler's attitudes

Please answer by ticking "X" on the corresponding answer.

	Agree	Uncertain	Disagree
I consider the food I prepare safe for consumers			
Training in food safety is essential to my work			
There is all the support that facilitates performing my job according to food safety principles			
Jewelry should not be worn in the kitchen			
Using cap, masks, protective gloves, and adequate clothing reduces the risk of food contamination			
The staff are provided with hand-washing sinks with soaps and paper towels.			
When cooking and reheating food, measuring internal food temperature is important			
Raw foods should be kept separately from cooked foods			
It is important to know the temperature of the refrigerator to reduce the risk of food safety			
It is not appropriate to thaw frozen food on the kitchen counter prior to preparation.			
We can keep ready to eat meat dishes and meat containing salads for longer than 2 hours at body temperature			
Improper storage of foods may be hazardous to health			

FAP/…….

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

Food-services staff with abrasion or cuts on fingers or hands should not touch unwrapped foods and use easily detectable plasters.			
Food handlers should not prepare food when coughing or having diarrhoea			
I believe that a sanitizing agent should be used to clean surfaces on which raw and cooked foods are prepared			
After handling raw meat or poultry, I should always wash my hands with soap and water.			

SURVEY

FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES OF FOOD HANDLERS

E. Constraints

What are the main barriers against applying food safety measures?

APPENDIX E

GENERAL ASSESSMENT CHECKLIST OF THE HYGIENIC CONDITIONS AND PRACTICES DURING VEGETABLE PREPARATION

		1	2	3		
		Adequate	Incomplete	Inadequate	N/A	Not Obs.
1	Is the premises looking in good repair with clean drains, clean walls, no peeling paint, no holes or gaps where pests might enter, evidence of pests etc.					
2	Is there a zoning in the food preparation facility?					
3	Is the received fresh procude stored in protected areas ?					
4	is the entrance to food service area controlled to staff only					
5	Are unprocessed raw vegetables prepared so that contamination and cross contamination does not occur?					
6	Is frozen food thawed properly?					
7	Are staff cleaning tools stored in appropriate manner and not at risk of contaminating food or equipment during preparation?					
8	Are floors, work surfaces, utensils and equipment clean ?					
9	All major pieces of equipment such fridges, freezers ovens, hot holding equipment, cold holding equipment are fitted with working temperature monitoring gauges					
10	Is there a washing sink designated for fresh produce only?					
11	are vegetable sanitizers made up correctly					
12	Are staff personal belongings stored in appropriate manner and not at risk of contaminating food or equipment during preparation?					
13	There are hand-washing facilities in food handling areas supplied with warm soap and disposable towels					
14	The Cleaning Schedule is visible					2
15	Where a chemical sanitiser is used are there records to show levels are maintained?					1
16	Are sanitisers for work surfaces readily available for use during food preparation?					
17	Waste containers are covered, kept clean					
18	Containers used to drain vegetables are kept clean					
19	Food handlers use gloves appropriately and correctly					
20	Kitchen personnel wear appropriate protective clothing and protective head coverings					
21	Hair are covered by allstaff in food preparation facility					
22	Visitors or unauthorized staff are granted protective clothing upon entry					
23	Correct use of equipment/ utensils / cutting boards for fresh produce to prevents cross-contamination					
24	Are food on hold covered					
25	Are there evidence of temperature control during storing?					
26	Are there evidence of temperature control during cooking?					
27	Are there evidence of temperature control during cooling?					

APPENDIX F

VISUAL ASSESSMENT COMPONENTS - CRITERIA

	Inspection component	Criteria
1	Are the premises looking in good repair with clean drains, clean walls, no peeling paint, no holes or gaps where pests might enter, evidence of pests etc.	**Incomplete**: Partially fulfilled requirements, evidence of pests, open to external environment, open drain, equipment or garbage bins are left dirty
2	Is there a zoning in the food preparation facility?	**Incomplete**: there is an attempt i.e. proper segregation for one area but lacking complete zoning, or only separate vegetable area yet receiving
3	Are received fresh vegetables stored in protected areas?	**Adequate**: clean baskets, elevated from floor, stored in clean cold rooms, stored separately from raw meat/poultry/fish.
4	Is the entrance to food service area controlled to staff only	**Adequate**: entry is permitted with protective clothing. Doors are kept closed
5	Are unprocessed raw vegetables prepared so that contamination and cross contamination does not occur?	**Adequate**: the vegetable preparation is area is kept clean, sanitized and separated from raw meat/poultry/fish. Use of separate utensils.
6	Is frozen food thawed properly?	**Adequate:** Thawing in cold rooms/refrigerator
7	Are staffs cleaning tools stored in appropriate manner and not at risk of contaminating food or equipment during preparation?	**Adequate**: Stored in separate areas from food production unit.
8	Are floors, work surfaces, utensils and equipment clean?	**Incomplete**: There is an attempt i.e. showing clean floors, partially clean surfaces, yet cutting boards have crevices; small/heavy equipment show dirt.
9	All major pieces of equipment such fridges, freezers ovens, hot holding equipment, cold holding equipment are fitted with working temperature	**Incomplete**: at least one refrigerator has no apparent temperature gauges or an internally fitted thermometer
10	Is there a washing sink designated for fresh produce only?	**Incomplete**: when the designated sink for washing vegetable is kept unclean and/or exposed to external environment
11	Are vegetable sanitizers made up correctly	Adequate (yes) or Inadequate (No)
12	Are staff personal belongings stored in appropriate manner and not at risk of contaminating food or equipment during preparation?	**Inadequate:** There is a clear evidence of staff belongings and clothing in food preparation are.
13	There are hand-washing facilities in food handling areas supplied with warm soap and disposable towels	**Incomplete**: There is no supply of soap or towel as shown during the visit or it is not functioning properly.
14	The cleaning schedule is placed and visible	Adequate (yes) or Inadequate (no)
15	Where a chemical sanitiser is used are there records to show levels are maintained?	Adequate (yes) or Inadequate (no)

16	Are sanitisers for work surfaces readily available for use during food preparation?	Incomplete: improper dilution or misuse
17	Waste containers are covered, kept clean	Adequate (yes) or Inadequate (no)
18	Containers used to drain vegetables are kept clean	Adequate (yes) or Inadequate (no)
19	Food handlers use gloves appropriately and correctly	Adequate (yes) or Inadequate (no)
20	Kitchen personnel wear appropriate protective clothing and protective head coverings	**Adequate**: complete protective clothing **Incomplete**: Staff wearing incomplete protective
21	Hair are covered by all staff in food preparation facility	**Incomplete**: when at least one of staff are permitted to facility without a hairnet or paper cap is used inside the production unit
22	Visitors or unauthorized staff are granted protective clothing upon entry	**Incomplete**: when only the hair net is requested upon entry.
23	Correct use of equipment/ utensils / cutting boards for fresh produce to prevents cross-contamination	**Adequate:** Proper color coded separation and proper use.
24	Are food on hold covered	Adequate or Inadequate
25	Is there evidence of temperature control during storing?	**Adequate**: Evidence of records
26	Is there evidence of temperature control during cooking?	**Adequate**: Evidence of records
27	Is there evidence of temperature control during cooling?	**Adequate**: Evidence of records

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